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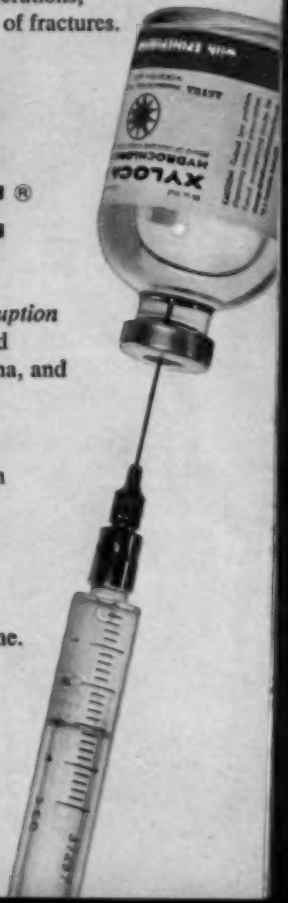

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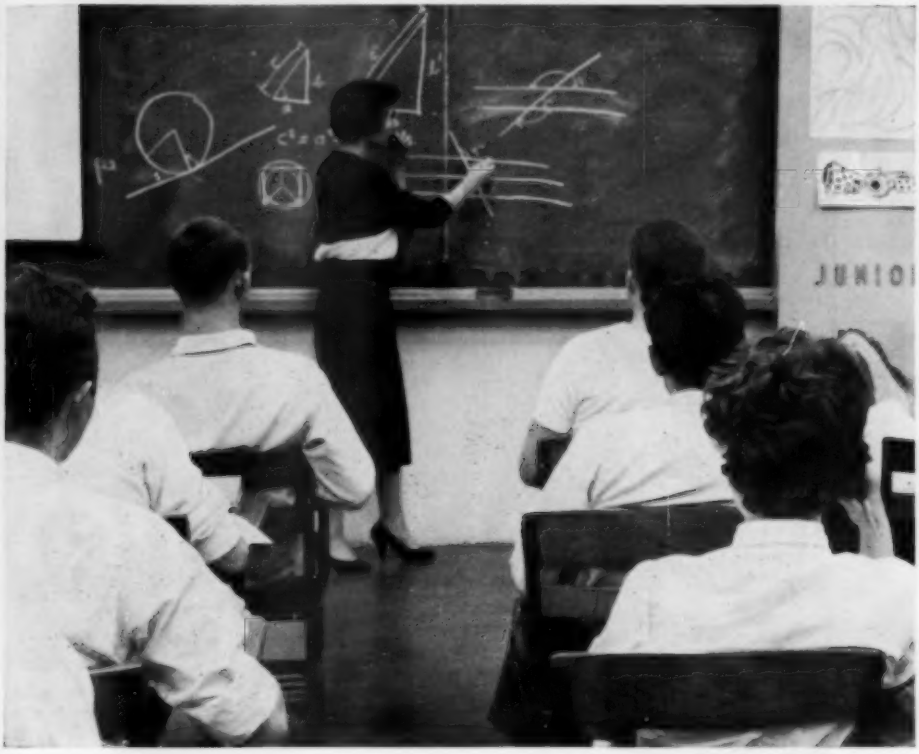

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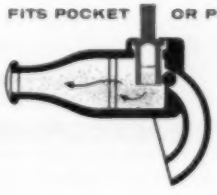

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1. Burrascano, J. J.: Sea View Hosp. Bull. *15*:149 (July) 1955.

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 75 *February, 1957* NUMBER 2

THE PATHOGENICITY OF ATYPICAL CHROMOGENIC MYCOBACTERIA FOR THE RHESUS MONKEY[1, 2]

L. H. SCHMIDT, RICHARD HOFFMANN, AND WILLIAM STEENKEN, JR.

(Received for publication August 30, 1956)

Introduction

This report deals with a preliminary study of the pathogenicities of two strains of chromogenic mycobacteria for the rhesus monkey. This investigation was a sequel to the more extensive studies of the biologic properties of these and of a third similar strain in mice and guinea pigs described by one of the writers in an accompanying paper (1). These more intensive studies had been stimulated by current reports (1–12) which have suggested that such atypical acid-fast bacilli may be the causative agents of human pulmonary disease.

Experimental

Procedures: The *M.B.* and *J.C.* strains, the origins and characteristics of which have been described elsewhere (1), were used in this study. Seven-day old subcultures of these strains in Dubos Tween®-albumin liquid medium, prepared from stock cultures on American Trudeau Society medium, provided the inocula for pathogenicity evaluations. The subcultures were diluted in 0.2 per cent bovine albumin to give the required inocula.

Each of the above strains was inoculated into a group of 8 rhesus monkeys. These animals ranged in weight from 3.1 to 6.2 kg., had been under laboratory observation for approximately four months, had exhibited repeatedly negative reactions to the intracutaneous injection of 0.05 ml. of a 1:10 dilution of Old Tuberculin (OT), and had normal chest roentgenograms one week prior to the beginning of this experiment. Pairs of these animals were inoculated intratracheally with 1 ml. of 10^0, 10^{-2}, 10^{-4}, or 10^{-6} dilutions of the respective strains. These inoculations and all roentgenographic examinations were performed under Surital® anesthesia. Postinoculation studies included roentgenographic examinations and measurements of tuberculin hypersensitivity at two, four, eight, and twelve weeks. In all postinoculation studies, 0.05 ml. quantities of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions of OT were employed in quantitating the degree of tuberculin hypersensitivity. In the four-, eight-, and twelve-week examinations, similar amounts of homologous strain tuberculin, prepared according to standard technique, were also employed. Surviving monkeys were sacrificed ninety-one days post inoculation, necropsies being performed

[1] From The Christ Hospital Institute of Medical Research, Cincinnati, Ohio; and the Trudeau Laboratory of the Trudeau Foundation, Inc., Saranac Lake, New York.

[2] This study was supported in part by a research grant, E 509, from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

TABLE 1

A SUMMARY OF THE PULMONARY DISEASE RESULTING FROM INTRATRACHEAL INOCULATION OF MONKEYS WITH THE M.B. AND J.C. STRAINS

Strain	Inoculum Number of Viable Units	Monkey Number	Reactions
M.B.	2×10^7	6808	Extensive infiltrate in right lung field two weeks post inoculation; marked clearing at four weeks; essentially normal lung fields at eight and twelve weeks; scarring and isolated caseous foci at autopsy.
	2×10^7	6809	Extensive infiltrate in right lung field at two and four weeks; marked clearing at eight weeks; nearly normal lung fields at twelve weeks; scarring and isolated caseous foci at autopsy.
	2×10^5	6810	Nothing remarkable at roentgenographic examinations and at necropsy.
	2×10^5	6811	Same as 6810.
	2×10^3	6812	Same as 6810.
	2×10^3	6813	Same as 6810.
	20	6818	Same as 6810.
	20	6820	Same as 6810.
J.C.	5×10^7	6815	Massive involvement in right lung field two weeks post inoculation; succumbed at 27 days; caseous pneumonia involving almost entire right lower lobe but limited to this lobe.
	5×10^7	6819	Moderate infiltrate in both lung fields two and four weeks post inoculation; marked clearing at eight weeks; succumbed to bowel injury at 64 days; clean-lined cavities in both lungs at autopsy.
	5×10^5	6821	Nothing remarkable at roentgenographic examinations and at necropsy.
	5×10^5	6822	Same as 6821.
	5×10^3	6773	Same as 6821.
	5×10^3	6777	Same as 6821.
	50	6806	Same as 6821.
	50	6807	Same as 6821.

aseptically. When lung and lymph node lesions were encountered, specimens were excised, homogenized in 0.2 per cent bovine albumin in a glass homogenizer, and aliquots of the homogenates were cultured on American Trudeau Society and 7H9 (13) solid media and in Dubos Tween-albumin liquid medium. The remaining lung structures were perfused with Zenker-formalin. After periods of fixation, washing, and hardening in alcohol, longitudinal sections of the lungs and tracheobronchial lymph nodes, approximately one-eighth inch thick, were prepared. Definite lesions and areas of uncertain character were excised, embedded in paraffin, sectioned, and stained with hematoxylin eosin or a Giemsa acid-fast stain used in this laboratory for the routine demonstration of mycobacteria in histologic preparations.

At the time the monkeys were inoculated, the numbers of viable units in the infecting doses and the capacities of the two strains to produce disease in guinea pigs were also determined. The numbers of viable units were estimated by plating 0.02 ml. quantities of 10^{-3} or 10^{-4} dilutions on 7H9 medium. Virulence for guinea pigs was checked by injecting

2 animals each, via the subcutaneous route, with 0.5 ml. quantities of the dilutions of culture employed for inoculating the monkeys. Body weight changes were recorded weekly. Measurements of tuberculin hypersensitivity (reaction to intracutaneous injection of 0.05 ml. of 1:10 dilution of OT) were made at four-week intervals. All guinea pigs were sacrificed twelve weeks post inoculation and subjected to gross pathologic examination.

Results

A brief summary of the roentgenographic and necropsy findings among the monkeys inoculated with the *M.B.* and *J.C.* strains has been set forth in table 1. As shown in this table, massive inocula of either of the strains (2×10^7 and 5×10^7 viable units, respectively) evoked significant pulmonary reactions.

One animal (No. 6815), inoculated with the *J.C.* strain, succumbed twenty-seven days post inoculation with a caseating pneumonia which involved in excess of two-thirds of the lower right lobe (*cf.* figures 1 and 4*A*) but none of the other lung structures. A second animal which received this strain and both monkeys which received the largest inoculum of the *M.B.* strain exhibited moderate amounts of pulmonary infiltrate on roentgenograms prepared two weeks post

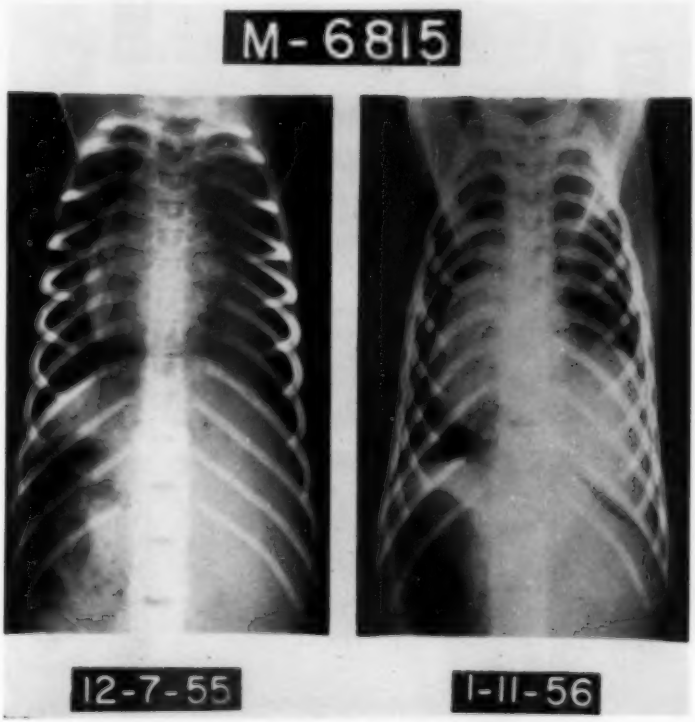

Fig. 1. Monkey 6815, inoculated with *J.C.* strain, 5×10^7 viable units. Preinoculation and terminal roentgenograms.

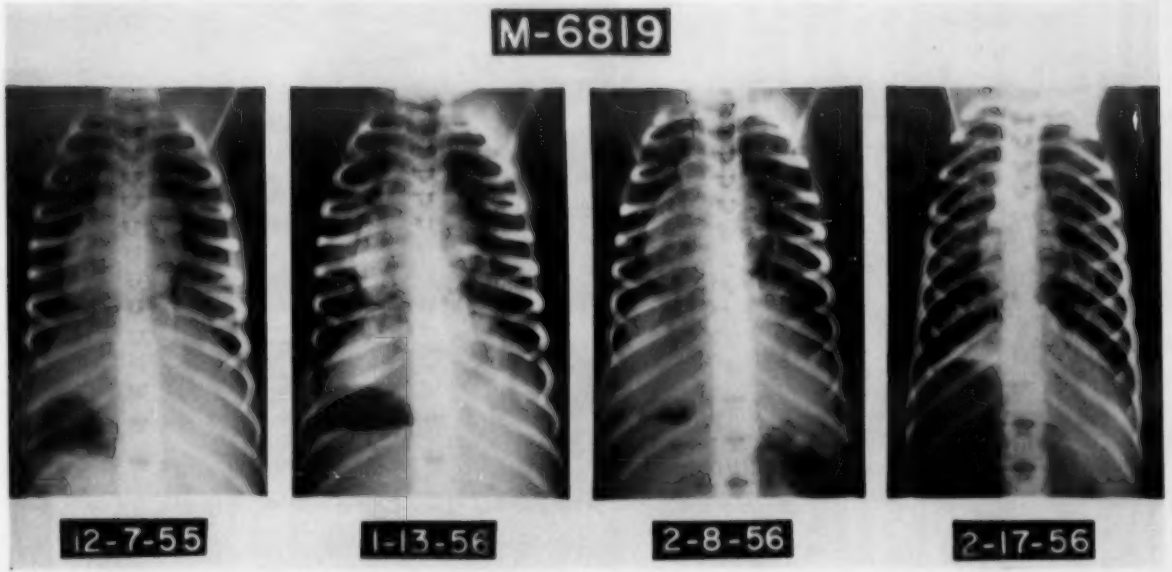

FIG. 2. Monkey 6819, inoculated with *J.C.* strain, 5×10^7 viable units. Preinoculation, four and eight weeks post inoculation, and terminal roentgenograms.

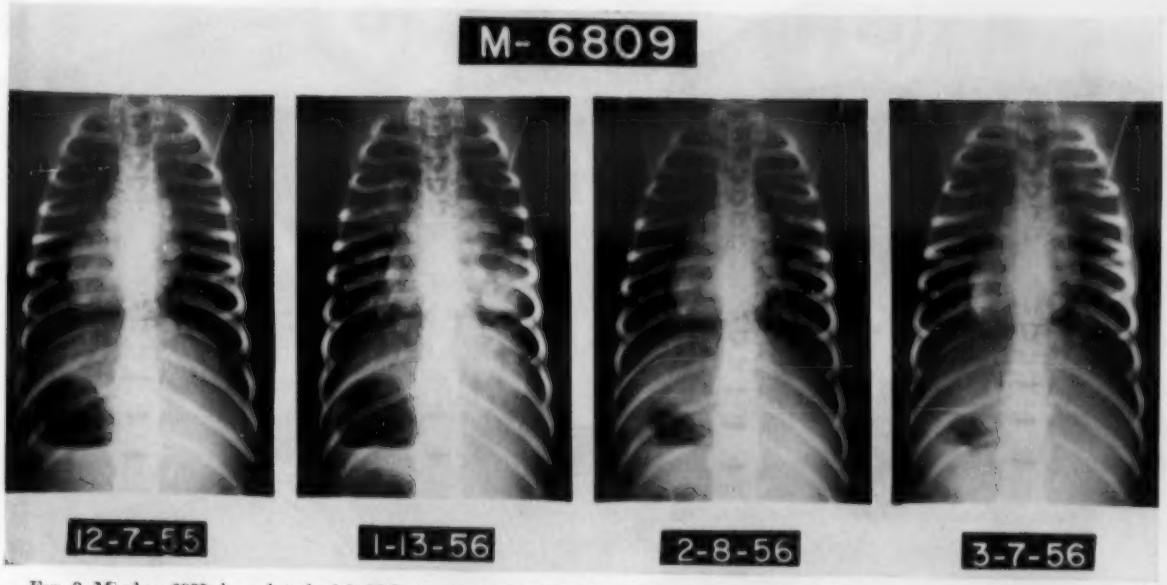

FIG. 3. Monkey 6809, inoculated with *M.B.* strain, 2×10^7 viable units. Preinoculation, four, eight, and twelve weeks post inoculation roentgenograms.

FIG. 4. *A* and *B*. Cut sections of fixed lungs of monkeys 6815 and 6819, inoculated with *J.C.* strain, 5×10^7 viable units.

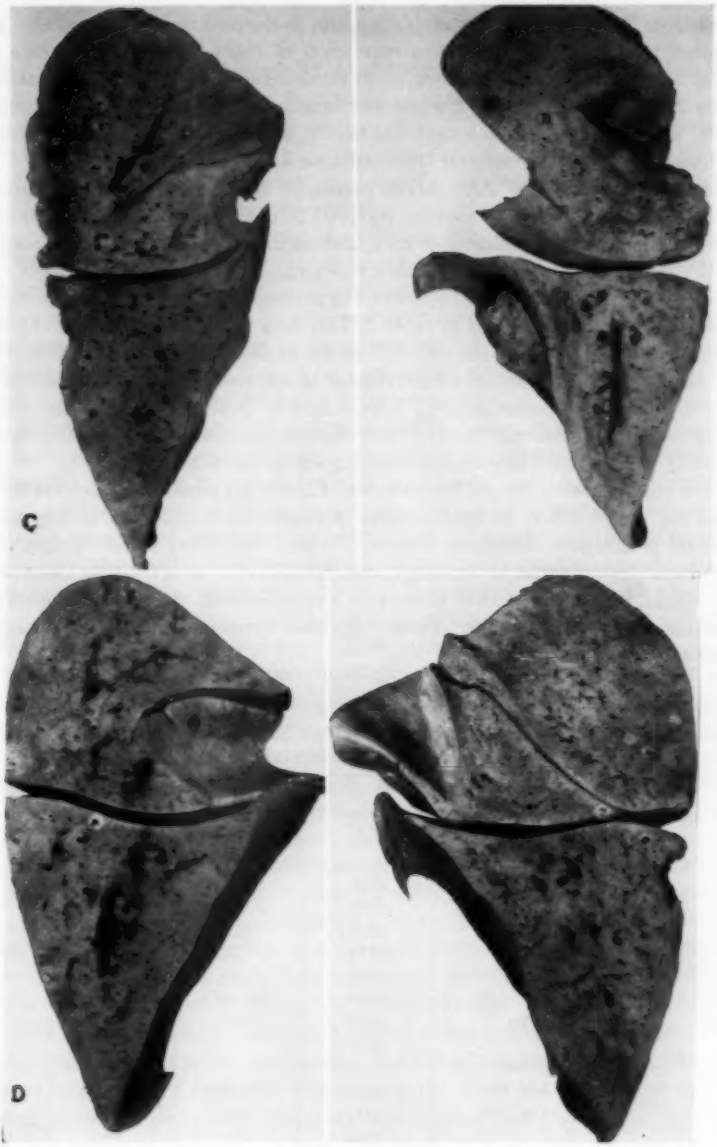

FIG. 4. *C* and *D*. Cut sections of fixed lungs of monkeys 6808 and 6809, inoculated with *M.B.* strain, 2×10^7 viable units.

inoculation. Repeat roentgenographic studies, performed two and six weeks later, indicated progressive, spontaneous regression of the infiltrate. Necropsies performed ninety-one days post inoculation revealed either clean-lined cavities (*cf.* figures 2 and 4*B*) or residual fibrosis and isolated foci of caseation (*cf.* figures 3 and 4*C* and *D*). Chromogenic acid-fast bacilli, seemingly identical to the organisms inoculated, were recovered from cultures of these lesions.

Neither the *M.B.* nor *J.C.* strain produced roentgenographic evidences of pulmonary disease in animals which received 2 to 5 $\times$ 10^5 or fewer viable units. Random selection of specimens of lung and regional lymph nodes at the autopsies of these monkeys yielded negative cultures.

The sequence of changes in tuberculin hypersensitivity (to OT) of the various animals has been summarized in table 2. The data therein suggest that the *M.B.* strain was more effective than the *J.C.* strain in inducing skin sensitivity. However, neither strain produced a high degree of reactivity and such sensitivity as was acquired was of comparatively brief duration. It is noteworthy that parallel comparisons of reactivity to OT and homologous-strain tuberculin showed uniformly weaker reactions to the homologous preparation.

A few comments on the pathogenicities of these atypical mycobacteria for the guinea pig are in order. In confirmation of earlier work (1), none of the inocula produced progressive, systemic disease. Massive caseation of the lymph nodes with ulceration occurred at the site of inoculation of 2 $\times$ 10^7 viable units of the *M.B.* strain. Smaller inocula of this strain and all inocula of the *J.C.* strain failed to induce even local reactions. Tuberculin skin hypersensitivity was evoked by all inocula of both strains.

TABLE 2

DEVELOPMENT OF TUBERCULIN HYPERSENSITIVITY IN MONKEYS INOCULATED WITH THE M.B. AND J.C. STRAINS

Strain	Inoculum Number of Viable Units	Monkey Number	Dilution of Tuberculin OT Evoking Reaction* Month of Observation		
			1	2	3
M.B.	2×10^7	6808	+1:100	±1:10	—
		6809	+1:100	+1:100	—
	2×10^5	6810	+1:100	±1:10	—
		6811	+1:10	+1:10	—
	2×10^3	6812	—	—	—
		6813	±1:10	+1:10	—
	20	6818	—	—	—
		6820	—	—	—
J.C.	5×10^7	6819	+1:100	±1:100	—
	5×10^5	6821	—	—	—
		6822	—	—	—
	5×10^3	6773	±1:10	—	—
		6777	—	—	—
	50	6806	—	—	—
		6807	—	—	—

* Dilutions of 1:10, 1:100, 1:1,000, and 1:10,000 of OT were employed in each test.

Discussion

The data set forth in this report suggest that the *M.B.* and *J.C.* strains of atypical, chromogenic, acid-fast bacilli have limited pathogenicity for the rhesus monkey. Only relatively massive inocula (2 to 5 $\times$ 10^7 viable units) produced roentgenographic and pathologic evidences of disease. In 3 of 4 monkeys which received such inocula, the disease was clearly self-limiting, with spontaneous regression of infiltrate on roentgenograms and pathologic residua comprising no more than clean-walled cavities or well-encapsulated foci of caseation. One of the animals receiving a massive inoculum of the *J.C.* strain succumbed. Although there was tremendous destruction of lung tissue in this monkey, there was striking limitation of disease to a single lobe and even in that lobe an unusually sharp demarcation between involved and noninvolved areas.

These reactions to the *M.B.* and *J.C.* strains are strikingly different from those observed in this laboratory following intratracheal inoculation of monkeys with typical human drug-susceptible tubercle bacilli. Large inocula of such strains (10^5 or more viable units) produce a rapidly fatal disease characterized by a fulminating caseating pneumonia which invariably involves three-fourths or more of the total lung structures. Smaller inocula (10 to 10^4 viable units) produce a steadily progressing disease which invariably terminates fatally sixty to one hundred and twenty days post inoculation, with massive cavitation and caseation.

The reactions of the monkey to the two chromogenic strains also differ from those which follow intratracheal inoculation of massive numbers of either the attenuated BCG or the avirulent H37Ra (unpublished observations in this laboratory). BCG has at most produced questionable infiltrate in 5 of 24 monkeys, whereas in 8 animals H37Ra failed to produce any reaction detectable by roentgenographic or pathologic examination.

The reactions to the *M.B.* and *J.C.* strains do have certain similarities to the sequence of developments which follows inoculation of monkeys with massive numbers of isoniazid-resistant catalase-negative tubercle bacilli (unpublished results in this laboratory). Certain of these strains initiate severe reactions detectable on roentgenographic examinations made two to four weeks post inoculation. These lesions subsequently undergo either essentially complete regression or leave residua of clean-walled cavities and small encapsulated caseous foci.

This comparison suggests that the pathogenicity of the two atypical chromogenic strains used in this study falls somewhere between that of typical human tubercle bacilli and that of well-known attenuated or avirulent mycobacteria such as H37Ra and BCG. There is a strong suggestion that, as in the case of isoniazid-resistant tubercle bacilli, the individual susceptibility of the host becomes an important factor in determining the reactions of the host to these atypical chromogenic bacilli. This may well explain both the rarity and occasional occurrence of disease with such organisms in man.

In concluding these comments, attention should be drawn to the comparative pathogenicities of the *M.B.* and *J.C.* strains for various laboratory animals. Because of differences in route of inoculation, it is difficult to make a strict comparison between the current results and those on guinea pigs and mice (1).

It is evident, however, that, irrespective of route of infection, relatively small inocula of these strains failed to produce progressive disease in guinea pigs or monkeys, whereas mice developed definite disease in both lungs and kidneys.

Summary

Two strains of atypical acid-fast bacilli have exhibited limited pathogenicity for the rhesus monkey. Intratracheal inoculation of 10^5 or fewer viable units provoked no evidence of disease. Inoculation of 10^7 viable units produced extensive pulmonary infiltrate detectable roentgenographically two weeks post inoculation. In all cases, the lesions were self-limiting anatomically and in 3 of 4 subjects underwent spontaneous regression.

Sumario

La Patogenicidad de las Micobacterias Cromógenas Atípicas para el Mono Rhesus

Dos cepas de bacilos ácidorresistentes atípicos han manifestado patogenicidad limitada para el mono rhesus. La inoculación intratraqueal de 105 o menos unidades viables no produjo signos de enfermedad. La inoculación de 107 unidades viables produjo extensa infiltración pulmonar, descubrible radiográficamente a las dos semanas de la inoculación. En todos los casos, las lesiones eran autolimitantes anatómicamente y en tres de cuatro sujetos experimentaron regresión espontánea.

Resume

Pathogénicité des mycobactéries chromogènes atypiques pour le singe Rhésus

Deux souches atypiques de bacilles acido-résistants ont démontré une pathogénicité limitée chez le singe Rhésus. L'inoculation intratrachéale d'un nombre égal ou inférieur à 105 unités viables ne déterminait aucun signe pathologique. L'inoculation de 107 unités viables provoquait des infiltrations pulmonaires étendues décelables sur les clichés radiographiques deux semaines après l'inoculation. Dans tous les cas, les lésions étaient anatomiquement circonscrites et trois sujets sur quatre ont présenté une régression spontanée.

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ATYPICAL CHROMOGENIC MYCOBACTERIA ASSOCIATED WITH PULMONARY DISEASE[1, 2]

Including a Report of Three Cases

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INTRODUCTION

Tuberculosis in man is a disease caused by the *Mycobacterium tuberculosis*, almost invariably of human or bovine origin. The ability to produce progressive disease in guinea pigs is one of the characteristics which distinguishes these bacilli (unless they are altered by drug therapy) from other mycobacteria. From time to time, however, reports have been published describing cases of human disease apparently caused by acid-fast bacilli which were nonpathogenic for guinea pigs. (For review of the earlier reports, *see* references 1–8, 12–16.) The papers of Buhler and Pollak in 1953 (1) and Timpe and Runyon in 1954 (2) marked the beginning of a period of recognition of such cases with increasing frequency (3–11).

Several investigators have made extensive studies of collections of atypical or chromogenic strains and compared them with mammalian tubercle bacilli and the saprophytic mycobacteria (2, 11–16). It is apparent, however, that the clinical significance of atypical acid-fast bacilli has been a problem since 1918 and that these strains for the most part have defied attempts at classification among the mycobacteria.

It is unfortunate that many reports do not discriminate between the isolated or single recovery of atypical acid-fast colonies from patients with all sorts of clinical conditions and the more significant instances of the *repeated* recovery of atypical mycobacteria from both diagnostic material and the diseased tissues in the absence of true tubercle bacilli. The former situation is difficult to interpret because of the possibility of laboratory error or contamination with the ubiquitous acid-fast saprophytes. Until more is known about the differential characteristics of the strains apparently associated with human disease, all chromogenic and/or guinea pig-avirulent mycobacteria isolated from patients with or without demonstrable disease should not be considered to belong in the same group.

A well-recognized source of strains of *M. tuberculosis* with attenuated virulence for guinea pigs is tuberculosis of the skin. Griffith (17) found that 99 of 140 cases of lupus yielded cultures which were not fully virulent for guinea pigs. Apparently none of these strains was pigmented and only 3 were considered to show "atyp-

[1] From the Trudeau Laboratory of the Trudeau Foundation, Inc., Saranac Lake, New York.

[2] This study was supported in part by a research grant, E36 C10, from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

ical" growth features. More recently, two well-defined guinea pig-avirulent strains (*M. ulcerans* and *M. balnei*) which cause ulcerating skin lesions in man have been described (18, 19).

The influence of light on the pigmentation of mycobacteria was mentioned in 1935 by Petroff and Steenken (20) and again in 1936 by Steenken and Landau (15). Baker (21) studied the phenomenon in greater detail in 1938 and found twenty-six non-mammalian strains with pigment formation which was light conditioned.

Recently, Runyon (11) has called attention to the fact that many of the atypical mycobacterial strains associated with pulmonary disease are photochromogenic or produce pigment (usually yellow) only on exposure to light.

Observations

Within a single year, 3 patients were seen at Trudeau Sanatorium who apparently had tuberculosis clinically but in whose sputum chromogenic, guinea pig-avirulent mycobacteria were found repeatedly and were also cultured from the lung tissue removed at operation. Two of the strains were yellow and photochromogenic; the other produced orange pigment even in the dark. These cases are also presented in detail.

Case Reports

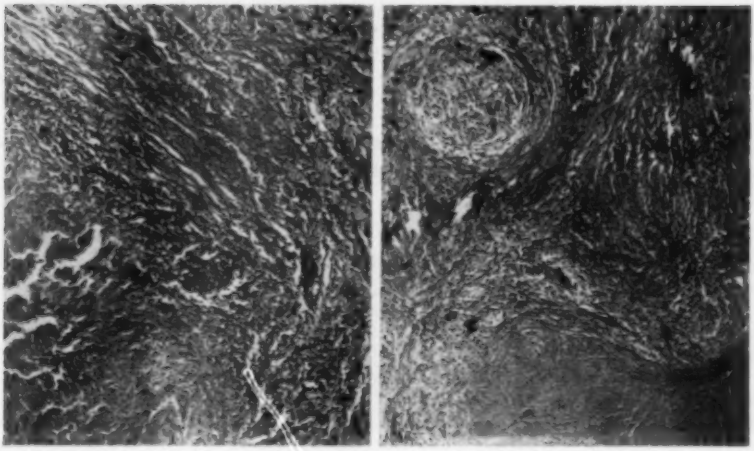
Case 1. J.C., a 49-year-old white salesman from Chicago, of Irish ancestry, was apparently well until June, 1953, when he noted fatigue, anorexia, and weight loss. There was no essential change in the chronic cough which he had had for many years (he was a heavy smoker) although there had been some increase in the mucopurulent expectoration. A duodenal ulcer had been diagnosed in 1931, and a partial gastrectomy was performed in 1948 after the complication of pyloric obstruction. Since that time the patient had been relatively free of ulcer symptoms but had remained underweight and slightly anemic. Chest roentgenograms in August, 1953, revealed dense shadows in the right upper lung field with apparent cavitation. There was no history of contact with tuberculosis or knowledge of the disease in the family. His wife was found to have a negative chest roentgenogram and a positive skin test with 1:10,000 Old Tuberculin (OT). He had no children.

On admission to Trudeau Sanatorium, September 3, 1953, the patient was thin and appeared chronically ill. Intracutaneous tests with 1:10,000 OT and first-strength purified protein derivative (PPD) were negative, but the test with second-strength PPD was positive. A chest roentgenogram (figure 1) showed dense shadows in the right upper third, and the presence of cavity was confirmed by planigram. A gastrointestinal series showed a gastroenterostomy with good function and no evidence of ulceration. The sputum contained moderate numbers of acid-fast bacilli on microscopy, and on culture yielded a moderately heavy growth of mycobacteria. The strain of mycobacteria was nonpathogenic for guinea pigs when inoculated subcutaneously, and the microbial colonies developed a bright yellow color on exposure to light. The microorganisms were longer and thicker than tubercle bacilli and contained many nonstaining vacuole-like areas, giving them the appearance of having acid-fast stripes. The growth was cream-colored if not exposed to light. The colonies were rather smooth and pasty and emulsified easily in saline. Growth appeared on inspissated egg medium in approximately two weeks at 37°C. There was less growth at 33°C. and none at room temperature. The organisms did not grow on plain Difco® nutrient agar. The culture was neutral red negative, catalase positive, and did not form cords. It was slightly resistant to streptomycin and to isoniazid *in vitro* and it was moderately to highly resistant to PAS. Similar mycobacteria were obtained from three subsequent sputum specimens.

The patient was treated with bed rest, modified by bathroom privileges. The maximal daily temperature was 99° to 99.6°F. for the first few weeks of hospitalization and was normal thereafter. Chemotherapy was begun on September 12, 1953, and consisted of isoniazid, 300 mg., and PAS, 12 gm., daily. Pneumoperitoneum was initiated two weeks later and produced only a moderate rise of the diaphragm. The patient gained weight steadily, and the acid-fast bacilli disappeared from the sputum on microscopy and culture after the third month of therapy. Serial roentgenograms showed progressive clearing, but

Case 1 (J.C.)

FIG. 1. (Upper left) Pretreatment chest roentgenogram taken on admission to Trudeau Sanatorium. Dense shadows in right upper third; cavity formation demonstrated in this area by planigram. (Upper right) After four and one-half months of chemotherapy, just before resection. There has been marked clearing of the disease, but cavity still seen on planigram. (Bottom) Two and one-half months after right upper lobectomy.



Case 1 (J.C.)

FIG. 2. Representative microscopic sections from excised lobe. (× 100) (Left) Edge of a large encapsulated caseous lesion, showing zone of epithelioid cell reaction between caseous material at lower left and fibrous scar at right. (Right) Epithelioid cell tubercle (left upper corner) in fibrous capsule of smaller caseous focus (lower right). There is a zone of epithelioid and giant cells around the focus.

planigrams confirmed the persistence of cavity. Bronchoscopy revealed normal airways. In January, 1954, streptomycin, 1 gm. daily, was added to the regimen.

Because of persistence of cavity after almost five months of therapy, a resection of the right upper lobe was performed in February, 1954. Grossly, the specimen was interpreted as showing caseous tuberculosis with cavity formation. There were numerous encapsulated or partially confluent caseous foci containing strands and dots of black pigment. Microscopic sections (figure 2) revealed numerous caseous foci with diffuse polymorphonuclear infiltration, nearly all of which showed a moderately thick zone or segments of epithelioid cell reaction. Most had well-developed fibrous tissue capsules containing slight lymphocytic infiltration and focal accumulations of black pigment. The remaining lung tissue in the vicinity of the caseous foci was replaced or distorted by large areas of fibrosis with black pigmentation and diffuse lymphocytic infiltration. Scattered here and there in the scar tissue were found partially organized epithelioid cell tubercles with giant cells.

Stained smears made from the necrotic tissue showed the same large acid-fast bacilli previously seen in the sputum, and cultures from the necrotic material as well as from the surrounding lung tissue yielded a moderate growth of yellow photochromogenic colonies which exhibited the same drug susceptibilities as those previously isolated from the sputum. Cultures on Sabouraud's medium and on blood agar incubated aerobically and anaerobically were sterile. Guinea pigs inoculated with emulsified caseous material and with homogenized lung tissue revealed no evidence of disease when killed four months later, but they did show positive reactions of limited extent to intracutaneous 5 per cent OT. On repeated testing these reactions were seen to reach their maximum in the second month and to diminish in intensity subsequently.

The patient's postoperative course was complicated by atelectasis of the right lower and middle lobes on the second postoperative day, necessitating bronchoscopy and subsequent tracheal aspirations. Thereafter, his convalescence was clinically uneventful. Roentgenograms showed satisfactory clearing of the postoperative reaction and good aeration of the

right middle and lower lobes, but an air pocket at the right apex remained. He had gained 15 pounds in weight by the time of his discharge against medical advice on April 29, 1954. He resumed full-time work at his previous job in June, 1954. Chemotherapy was terminated in March, 1955, approximately one year after resection. He was well and working in May, 1956.

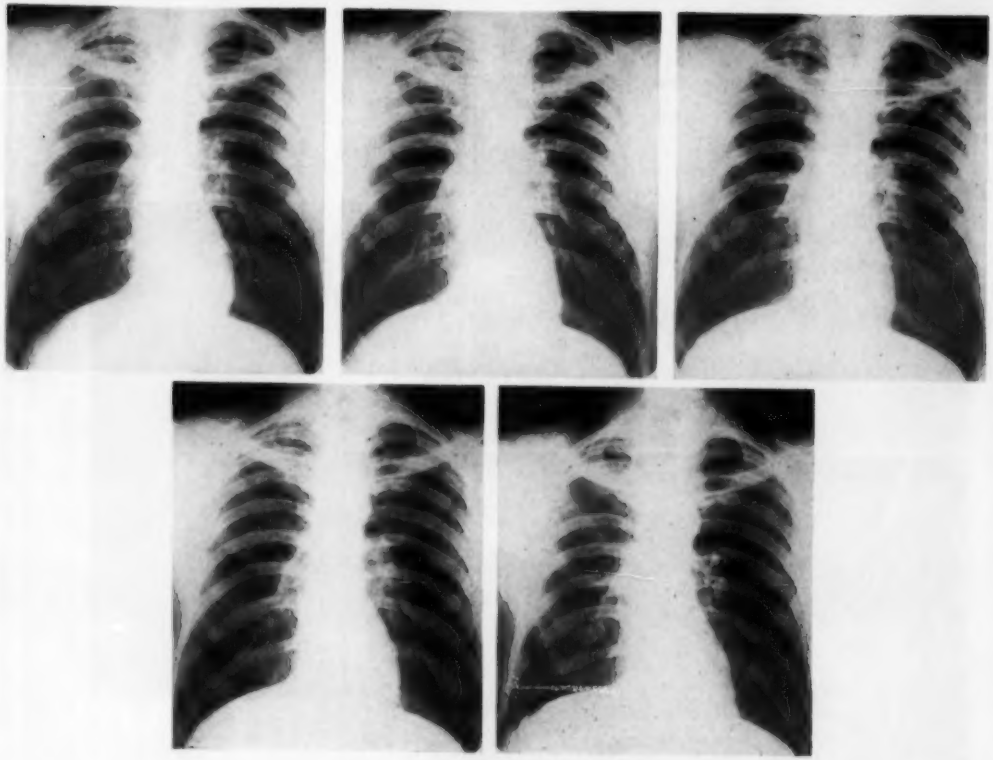
The next case was similar to the first, except that there was no antecedent gastrointestinal trouble and the time of onset of pulmonary disease could be determined more closely.

Case 2. M.B., a 46-year-old white banker from Philadelphia, of Scotch-Irish ancestry, had complained of mild hoarseness, phlegm in the throat, and easy fatigability for one year. Pulmonary tuberculosis was diagnosed in June, 1953, by chest roentgenograms (figure 3) during a voluntary medical check-up. A sputum specimen at that time contained small numbers of acid-fast bacilli. Treatment with modified bed rest, streptomycin, and PAS was started in August, 1953, approximately three weeks before admission.

A paternal grandmother had died of tuberculosis. A kidney stone had been passed in 1949. He had served in the United States Navy from 1941 to 1945 without service-connected injury, disease, or disability. A chest roentgenogram on discharge had been taken but could not be located. The patient's four children were found to have normal chest roentgenograms and, in addition, two of them had negative tuberculin skin tests. His second wife, whom he had married two months after diagnosis of tuberculosis had been made, was herself found to have a small circumscribed pulmonary lesion on routine chest roentgenogram four months before their marriage. This lesion was later resected and proved to be benign histoplasmosis. Her tuberculin skin test was negative.

The patient was admitted to the Trudeau Sanatorium in September, 1953. He did not appear ill, and no physical abnormalities were noted aside from a few adventitious sounds at the right apex. A Mantoux test with 1:1,000 OT was positive. The chest roentgenogram revealed abnormal densities with cavitation in the right apex (figure 3), confirmed by planigram. A concentrated sputum specimen did not show acid-fast bacilli on smear but yielded three yellow photochromogenic colonies on culture. This growth was similar in appearance to that described in *Case 1*, except that the colonies were not as smooth, were more crumbly, and did not emulsify in saline quite as easily. Other characteristics were also similar (*see* tables 1–4). The same atypical strain was isolated from the patient preoperatively on four different occasions, twice from sputum and twice from gastric washings. True tubercle bacilli were never found.

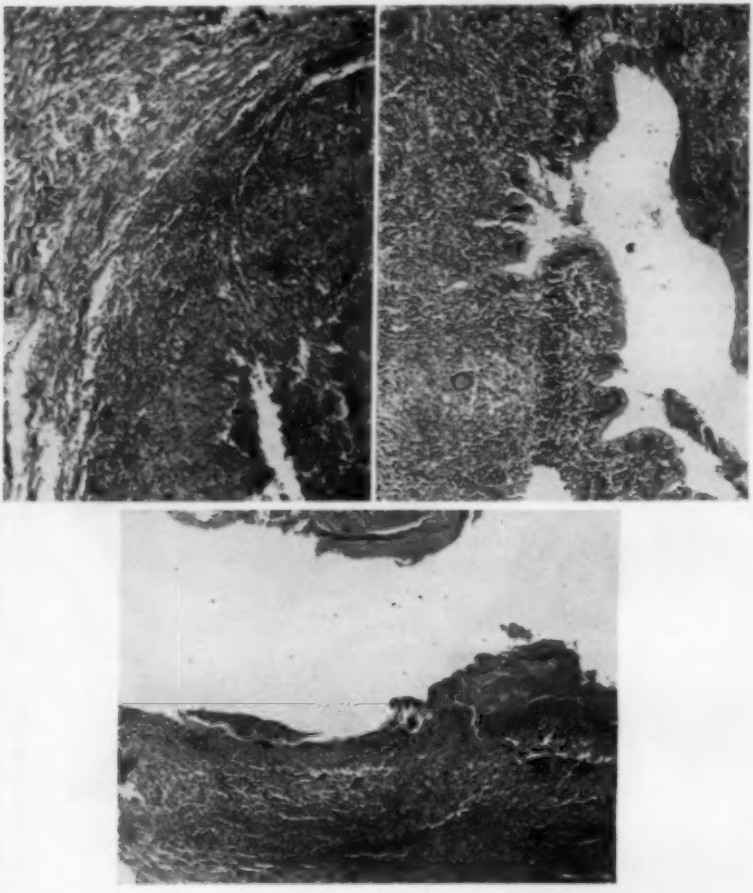
The treatment regimen was continued unchanged, with subsequent improvement both clinically and roentgenographically. A single mycobacterial colony grew from the culture of gastric washings on November 2, 1953, but the findings remained consistently negative thereafter despite persistence of the apical cavity. Isoniazid, 300 mg. daily, was added to the regimen on February 6, 1954. Because of persistence of cavity after almost six months of chemotherapy, resection of the right upper lobe was performed on February 16, 1954. The resected specimen contained many encapsulated caseous foci and one apical air-containing cavity. The peripheral bronchi were definitely involved in the disease process. The histologic diagnosis (figure 4) was chronic active fibroid and fibrocaseous pulmonary tuberculosis. In many areas the caseous material was seen to contain small accumulations of black pigment, and often the periphery of such lesions revealed diffuse polymorphonuclear infiltration. In some smaller foci a zone of granulation tissue was present between the caseous material and the fibrous capsules while, in others, the caseous material abutted directly on the fibrous tissue wall which was usually well developed and showed only slight lymphocytic infiltration. At one point, ulceration and necrosis of the mucosa of a bronchus were noted with epithelioid reaction at the point of ulceration. There were large areas of scar tissue arranged as thick, irregularly interlacing bundles of collagen with some foci of hyalinization.



Case 2 (M.B.)

FIG. 3. (Upper left) Chest roentgenogram obtained in June, 1953, when diagnosis of tuberculosis was first made. Infiltration and cavity at left apex and probable cavity in first anterior interspace. (Upper center) One month later, no change. (Upper right) On admission to Trudeau Sanatorium on September 21, 1953, after three weeks of chemotherapy. No appreciable change. (Lower left) After five and one half months of chemotherapy, just prior to resection. Apical cavity still visible, but clearing of disease elsewhere. (Lower right) July, 1954, five months after right upper lobectomy.

Acid-fast bacilli similar to those seen in the sputum were noted in the smears from the cavity contents as well as from two separate encapsulated caseous foci. Cultures from the cavity contents and from a caseous focus yielded a total of four chromogenic colonies similar in all respects, including drug susceptibility, to those found in the sputum. Guinea pigs inoculated subcutaneously with the same material developed transiently positive OT



Case 2 (M.B.)

FIG. 4. Representative microscopic sections from excised lobe. (× 100) (Upper left) Margin of encapsulated caseous focus with zone of granulation tissue between caseous material (right side) and fibrous capsule. (Upper right) A bronchus with diffuse cellular infiltration in the wall and a small tubercle with giant cell (lower left corner) in the submucosa. (Bottom) The apical cavity showing a rather smooth wall of dense caseous material, a narrow epithelioid cell zone, and dense fibrous capsule. At the top is a piece of caseous material, part of the opposite wall.

skin tests, but showed no evidence of disease on autopsy four months later. A heavy subcutaneous inoculation into 2 tuberculin-negative guinea pigs of the culture obtained from the lung tissue failed to produce evidence of disease in the animals, although again a transiently positive tuberculin test was noted. Cultures on Sabouraud's medium and blood agar aerobically and anaerobically were sterile.

The patient's convalescence was uneventful, and cultures of gastric washings following operation were negative for mycobacteria on several occasions. Seven months after resection the patient resumed part-time work; and five months later, full-time work. Chemotherapy was discontinued in February, 1955, one year after resection. The patient was well and working as of May, 1956.

The third case is different from the others in regard to the recovered strain of mycobacteria, which was scotochromogenic, and the history of the illness. The patient apparently had had pulmonary disease for many years and had worked in dusty atmospheres, but silicosis was not present in the resected lung.

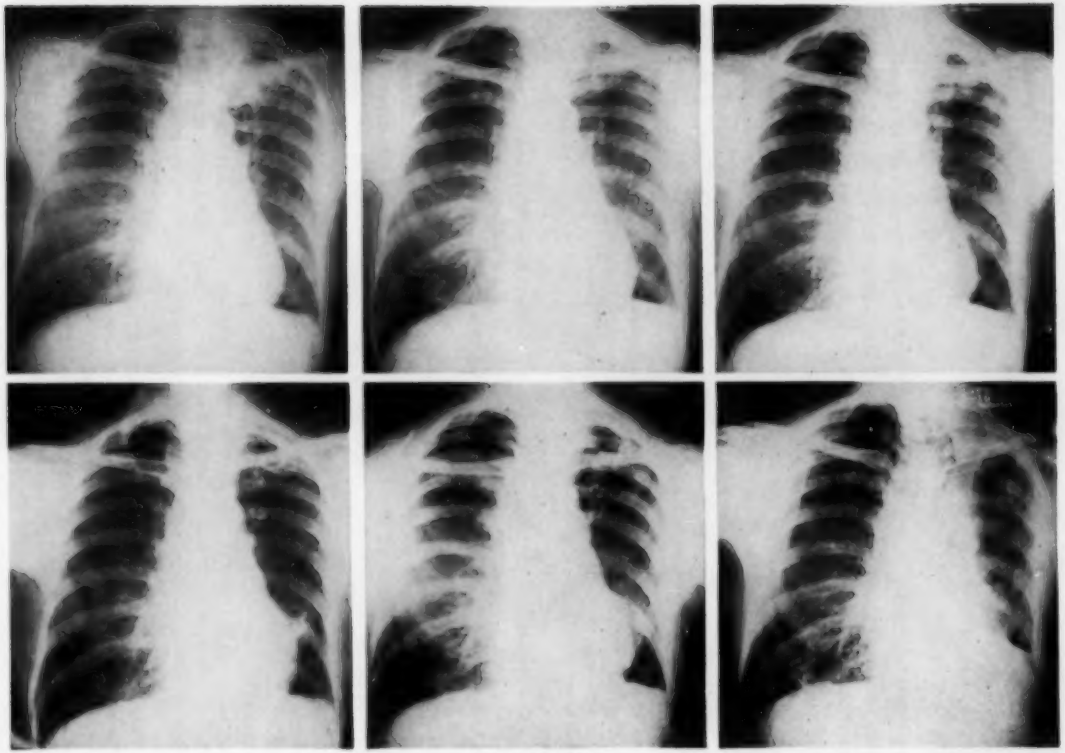
Case 3. L.C. was a 44-year-old white tool cutter and grinder, of English-Scotch ancestry, from Carbondale, Pennsylvania. He was sent to Trudeau Sanatorium by his local physician for diagnosis and recommendation. He first had symptoms referable to the respiratory tract at the age of eighteen when he had an hemoptysis supposedly a result of cardiac disease. In 1952, following another hemoptysis, gastric washings were reported as negative for acid-fast bacilli, but pulmonary disease was evident in the chest roentgenogram (figure 5). The patient continued working until he had another episode of bleeding in March, 1954, when the roentgenogram showed an increase of pulmonary disease with cavitation, and one of five sputum smears was positive for acid-fast bacilli. Treatment with streptomycin and PAS was started, but the patient resumed part-time work after only three months in a local sanatorium.

The patient had been exposed to silica and carborundum dust: He had worked as a coal miner from 1933 to 1940, a tool grinder from 1941 to 1944, and as a machine tool repair man from 1944 to 1954. There was no history of contact with tuberculosis. Four children were living and well.

On admission to the Trudeau Sanatorium in October, 1954, he was a thin man whose physical examination was negative except for a right inguinal hernia. He had a moderate cough productive of approximately 20 ml. of mucopurulent sputum daily. Chest roentgenograms showed linear exaggeration throughout the right side, a scoliosis to the right, mottling in the left upper third with a 2 by 3 cm. cavity in the apex, a small amount of mottling at the left base, blunting of the left sulcus, and displacement of the mediastinum toward the left side. Planigrams confirmed the presence of cavity in the apex. There had been some clearing of the process in the lower left half and a possible slight contraction of the cavity since the onset of chemotherapy six months previously.

A Mantoux test with 1:1,000 OT was negative, but a positive reaction developed with the 1:100 dilution. The sputum contained a moderate number of acid-fast bacilli on smear and, on culture, produced a moderately heavy growth of orange-pigmented smooth colonies. Identical findings were obtained from three sputum specimens approximately three days apart. This strain was scotochromogenic (11), that is, produced its pigment even in the dark. The organisms were pleomorphic, acid-fast cocco-bacilli. They were slightly resistant to streptomycin and isoniazid and moderately to highly resistant to PAS. Two guinea pigs inoculated subcutaneously with the pure culture developed a transiently positive tuberculin skin test but showed no evidence of progressive tuberculosis when killed three and one-half months later.

Resection of the left upper lobe was performed by Dr. Charles K. Kirby at the Hospital of the University of Pennsylvania in January, 1955, after ten months of chemotherapy. The writers are indebted to Dr. Kirby and to Dr. Joseph Aronson of the University of Pennsylvania for the following information on the pathologic and bacteriologic findings:



Case 3 (L.C.)

FIG. 5. (Upper left) Chest roentgenogram obtained in June, 1952, following hemoptysis. There is mottling in left upper third, blunting of left sulcus, and exaggeration of linear markings throughout both sides. (Upper center) Five weeks later, clearing on the left. (Upper right) Development of cavity in left apex, March, 1954. Sputum positive for acid-fast bacilli by smear; chemotherapy instituted. (Lower left) Increase in size of left apical cavity and evidence of disease in left lower lobe, September, 1954. (Lower center) On admission to Trudeau Sanatorium in October, 1954. (Lower right) May, 1955, after left upper lobectomy.

The specimen was firm, grayish-white in color, and was covered by a thickened pleura measuring approximately 2 mm. in thickness. A cavity 4 cm. in diameter contained a small amount of pultaceous reddish material and was surrounded by white fibrous tissue. There were also a few scattered caseous foci. Sections through the cavity wall showed foci of amorphous hyaline debris or caseation necrosis surrounded by endothelial cells and fibrous tissue, and foci of lymphocytic infiltration. Foreign-body giant cells were seen within the fibrous tissue layer, and new capillary growth and hemorrhage were visible in some areas near the cavity wall. Other areas of the lung revealed small foci of caseation surrounded by fibrous tissue, lymphocytes, and foreign-body giant cells. No diffuse black areas of lung were seen. There was no indication of silicosis in the excised lung.

Smears made from the cavity contents showed several small, short, thick, acid-fast bacilli, although none were demonstrable in the sections. A chromogenic acid-fast bacillus was obtained by culture of the material from the pulmonary cavity. The colonies were orange-colored even when grown in the dark. The culture was not pathogenic for guinea pigs.

The patient was discharged from the county tuberculosis hospital in July, 1955, and subsequently returned to full-time work as a machine operator. Chemotherapy was stopped in January, 1956, one year after the resection. As of May, 1956, the patient has remained well and working, and sputum smears have been negative for acid-fast bacilli.

Biologic Characteristics of the Strains

A summary of some of the biologic characteristics of the three atypical mycobacterial strains is given in table 1. It may be seen that the two photochromogens (strains *J.C.* and *M.B.*) resembled each other very closely, whereas the scotochromogenic *L.C.* strain differed in several respects. In regard to cord formation, neutral red test, and catalase activity, all three strains behaved like the known saprophytic mycobacteria. It is worthy of mention at this time that the catalase activity sharply differentiates both atypical and saprophytic mycobacteria from *Mycobacterium tuberculosis*. The former not only have a greater catalase activity when grown in ordinary medium, despite the fact that they are more or less resistant to isoniazid, but even the isolated colonies which appear in tubes of medium containing high concentrations of isoniazid still retain this activity. Furthermore, as mentioned in a previous report from this laboratory (22), when an atypical or saprophytic strain is made highly resistant to isoniazid by serial transfers in drug-containing medium, the increased catalase activity remains.

The results of *in vitro* susceptibility tests with a wide variety of drugs are indicated in table 2, along with those obtained with *M. tuberculosis* strain H37Rv and *M. phlei*.

The inoculum for each test was prepared by making a suspension in physiologic saline from a freshly growing culture on solid medium and diluting to the desired turbidity. Each strain was tested in duplicate, using the undiluted suspension and a tenfold dilution. One double loopful was spread onto the surface of the media slanted in test tubes. When solvents other than water were utilized to dissolve the drug, an amount of the solvent equal to that in the highest concentration of drug was incorporated into the control tubes.

It may be seen from the table that the atypical strains were less susceptible to the three antituberculous agents in general use than was the human H37Rv strain, but the increased resistance to streptomycin and to isoniazid was relatively slight. Resistance to viomycin and neomycin was also slightly increased

TABLE 1

SUMMARY OF BIOLOGIC CHARACTERISTICS

	J.C. (Strain 1)	M.B. (Strain 2)	L.C. (Strain 3)
Type of growth	Smooth, pasty, easily emulsified	Rough, more crumbly, emulsifies less easily	Smooth, flat, easily emulsified
Morphology of cells	Long, thick, beaded acid-fast bacilli	Long, thick, beaded acid-fast bacilli	Small, pleomorphic granular
Pigment			
in dark	none	none	orange
in light	yellow	yellow	orange
Growth			
at 37°C.	Good in 7–12 days	Good in 7–12 days	Good in 10–14 days
at 33°C.	Moderate in 16 days	Moderate in 16 days	Moderate in 16 days
at room temperature	Slight in 21 days	Slight in 21 days	Moderate in 21 days
on nutrient agar	0	±	+
Cord formation	Poor	Poor	Poor
Neutral red test	Negative	Negative	Negative
Catalase activity	Excess	Excess	Excess
Pathogenicity for mice intravenously	Moderate	Moderate	Unknown
Pathogenicity for guinea pigs:			
Subcutaneously	Avirulent	Avirulent	Avirulent
Intracardially	Very slight	Very slight	Unknown
Sensitivity of infected guinea pigs to Old Tuberculin	+(Transient)	+(Transient)	+(Transient)

over that shown by the H37Rv strain. Amithiozone and the thiocarbanilide compound (SU 1906) were interesting in that the two photochromogens were as susceptible to them as the H37Rv strain, whereas the *L.C.* strain and *M. phlei* were definitely more resistant. Cycloserine proved to be equally effective against all five strains tested, while pyrazinamide in medium at pH 5.3 was ineffective against all the strains except the H37Rv. The three atypical strains proved to be slightly more susceptible than the H37Rv to penicillin and erythromycin. Polymyxin B and bacitracin, not shown in the table, were found to be equally ineffective against all the strains.

The antifungal agents, stilbamidine, nystatin, actidione, Asterol,[3] filipin, and rimocidin were also evaluated against these cultures. None of them proved to be effective growth inhibitors.

The three atypical strains were incapable of producing progressive disease in guinea pigs inoculated subcutaneously even when the inoculum was very large. It will be noted from table 3, however, that large numbers of these mycobacteria

[3] Registered trade name of Hoffman-La Roche, Inc., for 2-dimethylamino-6-(beta-diethylamino-ethoxy)-benzothiazole dihydrochloride.

TABLE 2

IN VITRO SUSCEPTIBILITY TO DRUGS; γ OR UNITS PER ML. OF SOLID MEDIUM

Drug and Concentrations Used	J.C. (Strain 1)			M.B. (Strain 2)			L.C. (Strain 3)			H37Rv			*M. phlei*		
	R*	PR	S	R	PR	S	R	PR	S	R	PR	S	R	PR	S
Isoniazid† 0.1, 1.0, 10, 50	0.1	—	1	0.1	—	1	0.1	1	10	—	—	0.1	1	—	10
Streptomycin 2, 10, 50, 200	2	—	10	—	2	10	2	10	50	—	—	2	—	—	2
p-Aminosalicylic acid‡ 1, 10, 100	1	10	100	1	10	100	1	—	10	—	—	1	100	—	—
Viomycin 10, 50, 200	10	—	50	—	10	50	10	50	200	—	—	10	—	—	10
Neomycin 1, 10, 100	10	—	100	1	10	100	10	—	100	1	—	10	—	—	1
Amithiozone† 5, 10, 50	—	—	5	—	—	5	10	—	50	—	—	5	50	—	—
Thiocarbanilide (SU 1906)†, ‡ 5, 10, 25, 100	—	5	10	—	5	10	100	—	—	—	5	10	100	—	—
Pyrazinamide (pH 5.3)† 10, 25, 100, 500	100	500	—	500	—	—	500	—	—	—	—	10	100	500	—
Cycloserine† 20, 40, 100	—	—	20	—	—	20	—	—	20	—	—	20	—	—	20
Oxytetracycline 20, 100, 500	20	100	500	20	100	500	20	100	500	—	20	100	—	20	100
Chloromycetin 10, 50, 200	10	—	50	10	50	200	200	—	—	—	10	50	10	—	50
Penicillin G 10, 100	10	100	—	10	100	—	10	100	—	100	—	—	10	100	—
Erythromycin 1, 10, 100	1	10	100	1	10	100	1	10–100	—	100	—	—	1	10	100
Stilbamidine 10, 100	10	—	100	10	100	—	100	—	—	100	—	—	100	—	—

* R, PR, and S are abbreviations for resistant, partially resistant, and susceptible, respectively.

† Indicates that test was done on Steenken and Smith's inspissated egg medium. For all other tests the medium was Middlebrook's 7H9 oleic-acid–albumin agar.

‡ Ciba compound, 4-butoxy-4′-dimethylaminothiocarbanilide.

when inoculated intracardially into guinea pigs produced grossly visible internal lesions which tended to heal spontaneously. The self-limited granulomatous lesions noted sixty-four days after infection were essentially small collections of lymphocytes, macrophages, and epithelioid-like cells without necrosis or capsule formation, and were devoid of visible acid-fast bacilli. The large inoculum of 1 mg. (dry weight) intracardially, which represented about one billion viable units, produced death in all 6 animals within thirty-six days, but the guinea pigs showed no gross lesions except swollen spleens. The cause of death has not been determined.

Guinea pigs infected with all three of the atypical strains showed positive reactions to intracutaneous 5 per cent OT. These reactions were usually not severe and only rarely became necrotic, and they tended to lessen in severity as the infection healed.

Guinea pigs infected with tubercle bacilli from patients with tuberculosis and others infected with each of the two atypical strains, *J.C.* and *M.B.*, were tested intracutaneously with human OT and with tuberculin made by standard technique from strains *J.C.* and *M.B.* Reactions were noted with all tuberculin preparations without any evident specificity.

The two photochromogenic strains had definite pathogenicity for mice, as indicated in table 4. Large inocula injected intravenously produced a high mor

tality, although the survival time was much longer than one would expect for a virulent human strain. The most striking feature of the mouse infection was the extent of the lesions in the kidneys. In many of the mice that survived longer than forty days, both kidneys were almost completely replaced by large yellow-gray

TABLE 3

STRAINS J.C. AND M.B.: PATHOGENICITY FOR GUINEA PIGS INOCULATED INTRACARDIALLY
Survivors Killed After 64 Days

Strain	Inoculum	Number of Animals	Days Survival	Lesions
	mg.			
J.C.	1.00	3	25, 33, 36	None grossly except swollen spleens
	0.10	3	All 3 killed after 64 days	Occasional isolated tubercles in lungs, livers, lymph nodes. Subcutaneous nodules
	0.01	3	All 3 killed after 64 days	Tiny subcutaneous nodules and generalized slight enlargement of lymph nodes
M.B.	1.00	3	9, 11, 25	None grossly except swollen spleens
	0.10	3	All 3 killed after 64 days	Pinpoint gray lesions in lungs, small gray lesions (granulomas) in kidneys, subcutaneous nodules
	0.01	3	All 3 killed after 64 days	Small granulomatous lesions in lungs, livers, and kidneys, and subcutaneous nodules

TABLE 4

STRAINS J.C. AND M.B.: PATHOGENICITY FOR MICE INOCULATED INTRAVENOUSLY
Survivors Killed After 64 Days

Strain	Inoculum	Number of Mice	Days Survival	Lesions
	mg.			
J.C.*	1.00	3	40, 58, 1 killed after 64 days	Enlarged spleens and lesions in lungs and kidneys in all 3
	0.10	3	All 3 killed after 64 days	3/3 in lungs, 1/3 in kidneys
	0.01	3	All 3 killed after 64 days	3/3 in lungs, 1/3 in kidneys
M.B.*	1.00	3	3, 45, 1 killed after 64 days	None in 3-day death; other 2 enlarged spleens and lesions in lungs and kidneys
	0.10	3	31, 34, 1 killed after 64 days	All had enlarged spleens and lung lesions, 2/3 had kidney lesions
	0.01	3	All 3 killed after 64 days	All had few lesions in lungs, and 3/3 in kidneys
M.B.†	1.00	9	41, 55, 55, 57, 70, 71, 75, 2 killed after 116 days	All had lesions in lungs and kidneys

* C57 black mice.

† Swiss-Webster white mice.

raised abscess-like lesions which were teeming with acid-fast bacilli. Microscopically, the kidney lesions were seen to be dense collections of mononuclear cells with a small amount of central necrosis, occasionally surrounded by narrow zones of collagenous tissue. Branch (12) called attention to similar lesions in mice infected intraperitoneally with some atypical strains.

Mice infected intravenously with the *M.B.* strain and treated with pyrazinamide, 1.0 per cent in the diet for thirty days, died at the same rate and had the same amount of disease as the untreated controls, whereas the median survival time was greatly prolonged in the group treated with streptomycin.

The pathogenicity for rhesus monkeys of the two photochromogenic strains, *J.C.* and *M.B.*, was studied by Schmidt and his associates. Their results, reported in an accompanying paper (23), indicate that pulmonary lesions may be produced by intratracheal inoculation in this host, but only with very large inocula. With one exception, the lesions were self limited and healed fairly rapidly.

Discussion

Reports of well-documented cases of pulmonary disease apparently caused by atypical mycobacteria have been steadily mounting during the past four years. An increasing awareness of the situation, coupled with the more widespread use of cultural and animal inoculation methods since the advent of the chemotherapy era, has undoubtedly contributed to the more frequent recognition of these cases. It seems, however, that other factors may be involved. Aside from the 3 cases reported herein, and one other patient whose disease was caused by a nonchromogenic completely drug-susceptible but guinea pig-attenuated mycobacterium, no other instances of pulmonary disease related to atypical acid-fast bacilli had been detected among approximately 800 patient-strains of mycobacteria isolated and/or tested for drug susceptibility in this laboratory from 1947 through 1953. Furthermore, in Griffith's (17) study of 1,157 cases of various types of clinical tuberculosis other than lupus, he found only fifteen strains which he classified as attenuated in virulence for guinea pigs or rabbits, and thirteen of these came from scrofuloderma, cervical lymph node, or bone and joint lesions. Not one attenuated strain was found among 275 cases of pulmonary tuberculosis.

The coincidence of the widespread use of chemotherapy in tuberculosis with the increasing recognition of these unusual cases has naturally led to speculation on the role of the drugs in the emergence of atypical mycobacteria. Since many cases (at least 10 of the 17 from Kansas City (8)) were discovered prior to 1952, it is unlikely that isoniazid can be implicated. In addition, such strains have not appeared experimentally despite the exposure of *M. tuberculosis* in many laboratories to all sorts of drug combinations *in vitro* and in animals. The emergence of attenuated isoniazid-resistant catalase-negative mutants upon exposure to isoniazid is a different phenomenon unrelated to the one under discussion.

Some atypical strains may be ordinary saprophytes or variants thereof which have become established in human tissues by virtue of specific factors which lower host resistance. Products of chemical or bacterial inflammation may play a part in the establishment of such strains in the lung tissue of patients with

chronic esophageal obstruction causing repeated aspiration and of those with concomitant lung diseases, such as lipoid pneumonia or silicosis. Further studies in persons without coexisting disease are necessary to discover factors possibly related to loss of general or local tissue resistance.

A third consideration is that the strains represent saprophytic bacilli which have become established in old "burned-out" lesions originally caused by *M. tuberculosis*. The establishment of the atypical mycobacteria could have taken place while the tubercle bacilli were still present or after their disappearance. A well-documented history of this nature has yet to be described in man or in the experimental animal. For the many patients with relatively new and active lesions associated with the atypical mycobacteria, this theory is not valid. For instance, in *Case 2* described herein, although eight years had elapsed between a presumably normal chest roentgenogram and the finding of cavitary pulmonary disease with atypical mycobacteria in the sputum, the lesion was active microscopically and contained an open unhealed cavity. When admitted to the hospital, one of the patients described by Wood, Buhler, and Pollak (8) had a negative tuberculin skin test which converted to positive three weeks later. She had generalized miliary disease and meningitis, and her baby, who was born during the illness, died four weeks later with caseous pneumonia.

A fourth possibility is that the atypical strains, or perhaps a subgroup of them, are variants of mammalian tubercle bacilli. Unless it can be demonstrated with certainty that these strains are transmitted from man to man, or that some other reservoir is implicated, it must then be assumed that the variation occurs continually.

The contagiousness of patients harboring atypical strains is not known. No instance of contact infection has yet been reported, aside from the mother and baby mentioned above. The four children, aged seven to twenty-one years, of the patient in *Case 2* described herein were examined. All four had negative chest roentgenograms and two had negative Mantoux tests. In the cases reported by Feldman and collaborators (24) and by Young (6), the family contacts also remained tuberculin negative. Crow (10) found no evidence of contact infection in his large series.

Clinically, patients apparently infected with atypical strains cannot be differentiated with certainty from those with ordinary tuberculosis. In the 3 cases described herein, the disease did not respond to initial chemotherapy as well as the usual case of tuberculosis with disease comparable in extent. Despite some favorable roentgenographic changes, the cavities remained open after six months of therapy, and in *Case 3* the sputum continued to yield viable chromogenic acid-fast bacilli. It is interesting to note that this failure of full response, also noted in other cases (3, 4, 6, 8), occurred despite the relatively *slight* increase of *in vitro* resistance to streptomycin and isoniazid exhibited by the atypical mycobacteria as compared with the H37Rv strain.

The differentiation of atypical mycobacteria associated with pulmonary disease in man from *M. tuberculosis* may be made easily by virtue of their pigmentation, morphology, drug-susceptibility patterns, greater catalase activity, poor cord formation, negative neutral red reactions, and finally, by their lack of

pathogenicity for the guinea pig. They may be distinguished with certainty from isoniazid-resistant guinea pig-avirulent organisms by their catalase activity, negative neutral red and cording tests, lesser degree of resistance to isoniazid, and their pigmentation and morphology. The differentiation of atypical strains from the common saprophytic mycobacteria is not so definitive at the present time, although certain characteristics may be useful. The growth rate of the atypical strains when first isolated is usually slower than that of the saprophytes, especially at room temperature. For those strains that are photochromogenic, the dependence of pigmentation on exposure to light is a unique feature. Animal pathogenicity is characteristic, at least for the photochromogens. Fatal disease with extensive lung and kidney lesions may be produced in mice by intravenous or intraperitoneal inoculation of large numbers of bacilli, and progressive fatal disease may be caused in hamsters (25). Drug-susceptibility patterns seem to offer a promising lead, especially with regard to the greater susceptibility of the photochromogens to amithiozone and thiocarbanilide compounds. Finally, Parlett and Youmans (26) have demonstrated antigenic similarity between a group of atypical strains and *M. tuberculosis*, but no antigen-antibody reactions between saprophytic strains on the one hand and members of the atypical group and *M. tuberculosis* on the other.

M. ulcerans and *M. balnei* (27) produce characteristic superficial lesions in patients and in experimental animals. Their temperature requirements (19, 28) (optimal growth at 28° to 33°C.) set them apart from other atypical strains.

Summary

Three case reports are presented of patients with chronic, cavitary pulmonary disease from whose sputum and resected lung tissue chromogenic, guinea pig-avirulent, acid-fast bacilli were isolated repeatedly in the absence of typical tubercle bacilli. Two of the atypical mycobacterial strains were photochromogenic and the other was orange-pigmented even when grown in the dark. All three were poor cord formers, showed excess endogenous catalase activity, and did not bind neutral red; they were somewhat more resistant *in vitro* than the H37Rv strain to most of the available antituberculous agents with the exception of cycloserine and amithiozone.

The two photochromogenic strains were able to produce kidney lesions, progressive disease, and death when inoculated intravenously into mice, while guinea pigs inoculated intracardially with moderate numbers of these bacilli developed small granulomatous lesions which tended to heal spontaneously.

Characteristics which serve to differentiate the atypical mycobacteria associated with human disease from *M. tuberculosis* and saprophytic mycobacteria are discussed, as are the possible origins of the atypical strains.

Sumario

Atípicas Micobacterias Cromógenas Asociadas con Enfermedad Pulmonar: Comprendiendo Presentación de Tres Casos

Preséntanse 3 historias clínicas de sujetos con enfermedad pulomar cavitaria crónica, de cuyo esputo y tejido pulmonar resecado se aislaron repetidamente bacilos ácidorresistentes, cromógenos, avirulentos para el cobayo, sin encontrarse típicos bacilos tuberculo-

sos. Dos de las atípicas cepas micobacterianas eran fotocromógenas y la otra era de color anaranjado, aun cuando se cultivaba en la obscuridad. Las tres eran malas formadoras de cordones, revelaban excesiva actividad endógena para la catalasa y no fijaban el rojo neutro; *in vitro* eran algo más resistentes que la cepa H37Rv a la mayor parte de los agentes antituberculosos disponibles, con excepción de la cicloserina y la amitiozona.

Las dos cepas fotocromógenas se mostraron capaces de producir lesiones renales, enfermedad evolutiva y la muerte al ser inoculadas intravenosamente en los ratones, mientras que los cobayos a los que se inocularon intracardialmente cantidades moderadas de estos bacilos manifestaron pequeñas lesiones granulomatosas que tendían a la curación espontánea.

Se discuten las características que sirven para diferenciar las bacterias atípicas asociadas con enfermedad humana, del *M. tuberculosis* y de las micobacterias saprofitas, y también los posibles orígenes de las cepas atípicas.

Resume

Mycobactéries chromogènes atypiques associées à une affection pulmonaire: rapport sur trois cas

Présentation de trois observations de malades atteints de tuberculose pulmonaire cavitaire chronique, chez lesquels on a constamment isolé dans les crachats et les tissus pulmonaires réséqués des bacilles acido-résistants chromogènes avirulents pour le cobaye, à défaut de bacilles tuberculeux typiques. Deux des souches myocobactériennes atypiques étaient photochromogènes et l'autre avait une pigmentation orange, même lorsqu'elle était cultivée dans l'obscurité. Les trois souches ne donnaient pas une bonne formation de cordes, on observait une hyperactivité de la catalase endogène et l'absence de fixation du rouge neutre. *In vitro* ces souches étaient légèrement plus résistantes que la souche H.37Rv à la plupart des agents antituberculeux disponibles, à l'exception de la cyclosérine et de l'amithiozone.

Inoculées aux souris par voie intraveineuse, les deux souches photochromogènes étaient capables de produire des lésions du rein, une forme évolutive de la tuberculose et la mort, tandis que chez le cobaye, l'inoculation intra-cardiaque d'un nombre modéré de ces bacilles provoquait de lésions granulomateuses ayant tendance à guérir spontanément.

Les caractères permettant de différencier les mycobactéries atypiques intéressées dans l'affection pulmonaire humaine du *M. tuberculosis* et des mycobactéries saprophytes sont étudiés ainsi que les origines possibles des souches atypiques.

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A LIMITED CLINICAL, PATHOLOGIC, AND EPIDEMIOLOGIC STUDY OF PATIENTS WITH PULMONARY LESIONS ASSOCIATED WITH ATYPICAL ACID-FAST BACILLI IN THE SPUTUM[1,2]

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Introduction

In recent years there has been an increase in the number of published reports of the isolation from sputum of unidentified acid-fast bacilli which are usually nonpathogenic for the guinea pig. In addition to these reports, some investigators (1–3) have reported the isolation of typical acid-fast bacilli which are avirulent for the guinea pig. Several investigators have reported the isolation of atypical acid-fast bacilli from the sputum of persons with demonstrable pulmonary lesions, considered tuberculous, while others have reported such organisms isolated from patients with pulmonary disease considered nontuberculous (3–7). Wilson and Morton (3) reported seven cases with normal chest roentgenograms. It has been the opinion of some (3, 5) that these organisms are recovered from the sputum of tuberculous patients after the disease has become "apparently arrested" or probably inactive.

Beginning in 1950 the isolation of acid-fast bacilli with atypical cultural characteristics was noted at Battey State Hospital. In most instances it was soon noted that these organisms were nonpathogenic for the guinea pig. From 1950 through August, 1955, 69 patients were admitted, 65 of whom consistently showed only atypical acid-fast bacilli on sputum culture. These represent about 1 per cent of all admissions during these years. These organisms are not to be confused with acid-fast saprophytes, a number of which are seen in this hospital each year and have been completely disregarded as far as this study is concerned. As the years passed and more patients were added to the group harboring atypical bacilli, it was thought that a detailed study of these patients was warranted.

The purpose of this study was to try to determine if these patients differed in clinical, epidemiologic, or pathologic features from patients infected with typical *M. tuberculosis*. It was also deemed advisable to determine if any evidence could be found to establish these organisms as etiologic agents for producing pulmonary disease.

Material and Methods

Material: The material consisted of case records of patients treated at Battey State Hospital; surgical specimens of resected lung tissue of 7 patients; records of the State Director, Division of Tuberculosis Control, State of Georgia, and local county health departments in Georgia.

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[1] From Battey State Hospital, Rome, Georgia.

[2] Presented before the Medical Session, as part of *Section 1A*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 21, 1956.

Bacteriology and pathology: Selected material from sputum, as well as pus and homogenized tissue from resected lung material, was spread on slides and stained by the Ziehl-Neelsen method for microscopic examination which was reported according to a modified Gaffky scale. Sputum, gastric lavage sediment, and macerated pulmonary resected material were digested by agitating for ten minutes in a paint shaker after the addition of an equal quantity of 4 per cent sodium hydroxide. This was followed by centrifugation for fifteen minutes, neutralization of the sediment, and inoculation on four tubes of Lōwenstein-Jensen medium. The cultures were incubated in an upright position for six weeks at 37° C. after an initial period of eighteen hours in a slanted position. Studies for susceptibility to isoniazid and streptomycin were done from saline suspensions of cultures, using solid medium (Lōwenstein-Jensen) in which the particular drug had been incorporated in appropriate concentrations. Results were read at five to six weeks and recorded as number of colonies per tube up to 40, 1-plus, 2-plus, 3-plus, and 4-plus.

The strains cultured from the first 13 cases were intensively studied for virulence determination in guinea pigs, chickens, and rabbits. Random-bred guinea pigs weighing about 300 gm. each were injected subcutaneously in the right groin with two different amounts of culture material: 0.1 mg. and 5 mg. moist weight suspended in 1 ml. of saline. The growth was obtained from positive Lōwenstein-Jensen culture slants. All guinea pigs were autopsied at eight weeks. Chickens weighing approximately 1 kg. each were injected with 5 mg. moist weight of culture intraperitoneally. These were examined at autopsy at three months. Rabbits weighing about 1.5 kg. each were injected intravenously with 0.1 mg. moist weight of culture and were examined at autopsy at 3 months. Strains from some of the remaining 56 patients were also intensively studied for virulence in guinea pigs. In other patient-strains which were studied in guinea pigs, inocula of digested specimens of sputum, gastric lavage sediment, or treated surgical material were injected subcutaneously in the right groin and the guinea pigs were sacrificed at eight weeks. In 3 surgical cases, untreated surgical material was also inoculated into guinea pigs. All guinea pigs were tested with 5 mg. of Old Tuberculin (OT) prior to autopsy. Impression smears of organs showing infection were stained and examined for acid-fast rods, and subcultures of suspicious material were done. Many cultures were tested for cord formation and the reduction of neutral red.

Surgical specimens from 7 patients were submitted to the pathology department for gross and histopathologic study. Photographs were made of the lesions of all fresh specimens. Using sterile technique, material was removed from each specimen for study, which included microscopic examination, culture, and animal inoculation.

Clinical data: The hospital records of all patients studied were reviewed and data were obtained on clinical features. For epidemiologic studies, records in the state and local health departments were reviewed for available tuberculin sensitivity and the status of the chest roentgenograms of family contacts. Mortality data on discharged patients were obtained from the same source and verified by obtaining certified copies of death certificates from the Bureau of Vital Statistics.

Observations

General observations: An analysis of the 69 cases with respect to race and sex (table 1) revealed 19 white females (27.5 per cent), 41 white males (59.4 per cent), 2 Negro females (2.3 per cent), and 7 Negro males (10 per cent). The total number of Negro patients with tuberculosis admitted to this hospital since 1950 has constituted 41 per cent of all admissions, and the number of cultures done on them has been proportionally the same. Analyses of age incidence revealed only 2 patients less than thirty years old (table 2). The incidence was much greater in those older than forty and reached its peak (28.9 per cent) in the sixty- to sixty-nine-year age group. Only 17.4 per cent of all patients having atypical cultures

TABLE 1

RACE AND SEX

	Number of Patients	Per Cent
White females	19	27.5
White males	41	59.4
Negro females	2	2.8
Negro males	7	10.0
Total	69	99.7

TABLE 2

INCIDENCE BY AGE

Age	Number of Patients	Per Cent
0–19	1	1.4
20–29	1	1.4
30–39	10	14.4
40–49	19	27.5
50–59	15	21.7
60–69	20	28.9
70–79	3	4.3
Totals	69	99.6

TABLE 3

FAMILY HISTORY

	Number of Patients	Per Cent
Family history positive for tuberculosis	12	17.4
Family history questionable for tuberculosis	2	2.8
Family history negative for tuberculosis	55	80.0
Totals	69	99.2

were less than forty years of age on admission. Of the total admissions of all tuberculous and suspected tuberculous patients since 1950, 50 per cent were less than forty. The occupational history and geographical distribution in Georgia were studied, but no significant observations were noted.

The chief complaint of the patients on admission to the hospital was cough in 46 per cent; and 39 per cent stated that their present illness had started with cough. The family histories were studied (table 3). The family was taken to include only father, mother, brothers, sisters, and children if the patient had any such relations. There were 55 patients (80 per cent) with a negative family history for tuberculosis. The marital history of 63 married patients was 100 per cent negative for tuberculosis. In no instance was there more than one known atypical patient per family. A study of past histories of all patients revealed that the inci-

TABLE 4

CUTANEOUS REACTION TO TUBERCULIN

	Number of Patients	Per Cent
Negative to 0.01 mg.	22	31.8
Negative to 1.0 mg.	1	1.4
Positive to 0.01 mg.	40	57.9
Positive to 0.1 mg.	6	8.6
Totals	69	99.7

dence of previous respiratory disease (one or more per patient) was high, there being 66 instances in 69 patients. Pneumonia had occurred in 34.7 per cent, and a history of malaria was noted in 33 per cent, but the latter disease had usually occurred many years previously when malaria was still highly prevalent in some sections of Georgia. There were 14 patients who had had a previous diagnosis of pulmonary tuberculosis. In only one had the diagnosis been confirmed by the isolation of typical *M. tuberculosis*. Two patients had been diagnosed by signs and symptoms and 11 by sputum microscopy and roentgenographic examinations. Five patients had had streptomycin and none had received isoniazid. The one patient diagnosed by typical culture had had no chemotherapy. The duration of illness prior to hospitalization ranged from zero months to thirty-one years. There were 4 patients who were asymptomatic when their disease was discovered by routine roentgenogram.

The duration of illness was less than six months in 5 patients and ranged from six to twenty-four months in 30 patients. Most of the other patients had been ill for many years with complaints of cough, chest pain, weakness, or dyspnea. Only one patient was acutely ill on admission. She was in status asthmaticus and had no definite infiltrative shadows on her chest film. She had cultures positive for atypical acid-fast bacilli and her skin test showed a 3-plus reaction to first-strength OT.

There were 22 patients (32 per cent) who failed to react to first-strength OT (table 4). No patient was too ill or toxic to be anergic to tuberculin, and no other recognized condition existed that would cause anergy. One patient with far advanced, cavernous disease, was negative to 1 mg. of OT. There were 33 related intrapulmonary complications, one or more per patient. Emphysema was the most common, while thickened pleura and atelectasis were next in order of frequency. No patient showed any evidence of extrapulmonary tuberculosis.

Roentgenographic findings: Roentgenographic evidence of pulmonary disease was noted in 67 patients (table 5). One patient with a negative roentgenogram had a bronchoscopic diagnosis of tuberculous endobronchitis, and another had a bronchographic diagnosis of bronchiectasis. The majority of patients had roentgenographic evidence of active pulmonary tuberculosis, while a few had chest films not entirely characteristic of this disease. There were 44 patients (64 per cent) whose disease was classified as far advanced, and in 17 (24.5 per cent) the

TABLE 5

ROENTGENOGRAPHIC CLASSIFICATION OF PULMONARY TUBERCULOSIS ON ADMISSION TO HOSPITAL

	Number of Patients	Per Cent
Far advanced	44	63.7
Moderately advanced	17	24.6
Minimal	2	2.8
Suspicious for tuberculosis	1	1.4
Tuberculous bronchitis	1	1.4
Chronic suppurative disease (lungs)	1	1.4
Bronchiectasis	1	1.4
Nontuberculous	1	1.4
Pleural effusion	1	1.4
Totals	69	99.5

TABLE 6

CHARACTERISTICS OF CULTURES

Strains noted		
Chromogenic	3	
Rough, rapidly growing	2	
Smooth, buff-colored	64	
Total	69	
Consistently atypical	65	(94%)
Converted *A* to *T*	2	
Converted *T* to *A*	1	
Alternately *A* and *T*	1	
Total	69	

disease was classified as moderately advanced. Most of the patients with far advanced classifications had demonstrable cavitation.

Bacteriologic findings: The cultures isolated from the patients in this study were of three different types or strains (table 6).

There were 64 patients whose cultures showed the same strain. On stained smears, the morphology of these organisms was often shorter and more coccoid than the acid-fast rods of typical *M. tuberculosis* but could not reliably be distinguished from them. On Löwenstein-Jensen culture slants the culture growth from each of these 64 patients was characterized by its dysgonic quality, slow growth, and moist, smooth appearance. These cultures were frequently covered with a buff film which was composed of tiny colonies with an occasional large colony amidst the pasty growth. Color varied from buff to light orange which became more pronounced as the cultures became older. Exposure to light did not seem to change this color appreciably.

There were 3 patients whose cultures were more chromogenic. These were

identical with the characteristics given above except that the degree of coloration was more pronounced and approached lemon yellow.

Two patients had cultures that were rough, dry, friable, and identical in color with *M. tuberculosis*. However, the growth rate was more rapid, and colonies at one week were often larger than those produced by *M. tuberculosis* at six weeks. This growth showed no resemblance to that exhibited by acid-fast saprophytes which are usually chromogenic, moist, smooth, and exhibit a very rapid growth rate.[3]

Of the 69 patients studied, specimens derived from 65 (94 per cent) revealed only atypical organisms on culture and the isolations were usually made repeatedly (table 6). There were 2 patients whose strains were originally atypical on culture whose sputum afterwards yielded only typical organisms on culture. One patient who originally had been discharging typical strains subsequently yielded only atypical mycobacteria. One patient's strains were alternately typical and atypical on culture. These 4 patients cannot be classified as having consistently atypical strains. Only one patient was diagnosed as having an atypical strain from a positive gastric culture alone. One patient-strain was classified as atypical on the basis of cultures of a resected surgical specimen; 8 positive atypical cultures were obtained from different areas of a resected lung specimen. This patient's sputum had been initially positive on direct smear, but no culture had ever been done. The patient had been admitted to the hosptial prior to the time when cultural studies were routinely done on all patients with sputum positive for acid-fast bacilli on microscopy. Specimens from nearly all patients were positive on direct smear, and it was noted that the Gaffky count on these smears was usually low and rarely over Gaffky V. Studies of the drug susceptibilities of the atypical organisms to streptomycin and isoniazid prior to any antimicrobial therapy showed that the strains from 52.6 per cent of 19 patients were partially or completely resistant to isoniazid and the strains from only 12.5 per cent of 32 patients were partially or completely resistant to streptomycin (table 7). Unfortunately, drug-susceptibility studies were not done on many patients prior to chemotherapy.[4] Studies on many positive atypical cultures for cord formation

[3] Following a recent communication from Dr. Ernest H. Runyon, Director of Laboratory Service, Veterans Administration Hospital, Sunmount, New York (14), the bacteriologic characteristics of the atypical cultures from the present series of patients have been reviewed and compared with the criteria for classification of atypical acid-fast bacilli as stated in the Veterans Administration-National Tuberculosis Association Cooperative Study of Mycobacteria (15). This classification is based on chromogenicity of cultures with reference to light conditioning. The cultures from 64 of the patients recorded in table 6 as smooth, buff-colored meet the criteria for classification as non-photochromogens. The three strains listed as chromogenic meet the criteria for classification as scotochromogens. The two other strains listed as rough, rapidly growing remain unclassified. No cultures resembling photochromogens were seen in this series of patients.

[4] A recent study of 22 patients not in the original series revealed atypical organisms which showed no instance of resistance to streptomycin in 14 cases, but 86.6 per cent of 15 patients revealed organisms which were partially or completely resistant to isoniazid. None of these patients had received isoniazid prior to drug-susceptibility studies and, from the records available, it seems reasonable to assume that none were contacts of patients previously treated with isoniazid.

TABLE 7

DRUG RESISTANCE PRIOR TO CHEMOTHERAPY

	Streptomycin		Isoniazid	
Susceptible	28		9	
Partially resistant	3	12.5%	4	52.6%
Resistant	1		6	
Totals	32		19	
Not done	37		50	
Criteria	Partially resistant		Resistant	
Streptomycin	2 to 10 γ		4 to 10 γ	
Isoniazid	2 to 5 γ		4 to 5 γ	

TABLE 8

ANIMAL INOCULATIONS

Cases with One or More Animal Inoculations, 47 (68 per cent)

	Negative	Per Cent	Limited Disease	Per Cent	Progressive Disease	Per Cent
Animal inoculation from culture 5 mg. inoculum	5		15		1	
Standard inoculum	2		2		1	
Animal inoculation from gastric specimen (culture positive)	9		1		0	
Animal inoculation from gastric specimen (culture negative)	6		0		0	
Animal inoculation from surgical specimen (culture positive)	2		0		0	
Totals	24	51	21	44.6	2	4

Note: Since this paper was completed, one of the patients whose strain had not previously been studied in animals yielded a surgical specimen which was positive for mycobacteria on culture but was negative on animal inoculation. A second patient whose strain is listed in this same category yielded a surgical specimen which produced limited disease on animal inoculation.

and reduction of neutral red were done, but the results were inconclusive. The sputum of several patients was studied for other pathogens, including pyogenic organisms and fungi, but nothing was found other than the expected normal flora.

Animal virulence studies: Atypical cultures isolated from the first 13 patients were studied extensively with respect to virulence for guinea pigs, chickens, and rabbits. No culture from any patient was studied for mouse virulence. There was no instance of infection in either chickens or rabbits at autopsy. As for the results of guinea pig virulence studies, one or more guinea pig inoculations were done from specimens of 47 patients (68 per cent) (table 8). Only two patient-strains produced disease in guinea pigs which at autopsy showed a progressive character similar in extent to that usually caused by typical organisms. One of

TABLE 9

DISAPPEARANCE OF MYCOBACTERIA FROM THE SPUTUM WITH CHEMOTHERAPY

	Number of Patients	Per Cent
Chemotherapy alone	17	30.3
Chemotherapy and surgery	7	12.5
Sputum remained infectious*	32	57.0
Totals	56	99.8

* Seven (12.5 per cent) of these were discharging atypical mycobacteria at the time of death.

these animals had been inoculated with a 5 mg. inoculation from a definitely positive atypical culture, and the other animal had received a 0.1 mg. inoculation from a positive atypical culture. There were 21 patients (44.6 per cent) in this group with strains which were inoculated into guinea pigs which showed only very limited evidence of disease at autopsy. The evidence of infection in these animals usually consisted of an enlarged lymph node, a small solitary abscess, or very minimal evidence of localized infection. Cultures of organs showing infection yielded the same atypical growth seen on initial cultures. It was also noted that this bacterial growth was sometimes recovered from organs that showed no gross evidence of disease. Of the 21 patient-strains which produced localized limited disease in guinea pigs, the inoculations in 15 cases were made with 5 mg. of culture material from definitely positive atypical cultures. There were 24 patient-strains (51 per cent) in this group which showed no evidence of even limited disease on guinea pig inoculation. In 7 of these 24, the guinea pigs were inoculated from definitely positive cultures, and 5 of the 7 were high-dosage inoculations of 5 mg. of culture material. Of the remaining 17 patient-strains which produced no disease in guinea pigs, all were tested in the animals by the routine inoculation of gastric or surgical specimens. Cultures of the same specimens at the same time were positive in 11 instances and negative in 6 cases.

Incidence of sputum "conversion" with chemotherapy: Fifty-six patients were given antimicrobial therapy, 17 (30.3 per cent) of whom "converted" their sputum (by culture) for three months or longer (table 9). The duration of drug therapy varied from three to fifty-seven months, and the average duration was seventeen months. Only 2 patients were observed on drugs for as short a time as three months. Seven patients who received drug therapy died while still discharging mycobacteria. There were 7 patients (12.5 per cent) on drug therapy who did not "convert" their sputum until after chest surgery, and these were counted as surgical "conversions" rather than chemotherapeutic "conversions." Evaluation of response to chemotherapy was not carried beyond surgery. Of the remaining 13 patients in the total series of 69, treatment evaluation was not done either because they received no chemotherapy or because they had had less than three months' hospitalization. However, none of these patients achieved sputum "conversion." In the group of patients studied for chemotherapy response, 32 of

TABLE 10

ROENTGENOGRAPHIC RESPONSE TO CHEMOTHERAPY

	Number of Patients	Per Cent
No change	13	23.2
Slight improvement	18	32.0
Moderate improvement	4	7.0
Marked improvement	7	12.5
Worse*	14	25.0
Totals	56	99.7

* Seven of the patients whose lesions showed progression ultimately died, 5 of whom died of progressive pulmonary tuberculosis (atypical).

Criteria:

0–25% clearing—slight improvement
25–50% clearing—moderate improvement
More than 50% clearing—marked improvement

the 56 had far advanced lesions roentgenographically, with extensive anatomic destruction of one or both lungs. In addition, 18 of the 56 represented unsanctioned discharges from the hospital on one or more occasions, but a few patients continued to receive drugs at home. Correlation of sputum "conversion" with drug regimen was attempted, but the results were inconclusive due to extreme variations in therapy and the small number of patients receiving each regimen. There was no conclusive evidence that *in vitro* resistance to isoniazid or *in vitro* susceptibility to streptomycin were reflected clinically when these drugs were used.

Roentgenographic response to chemotherapy: The 56 patients given antimicrobial therapy for three months or longer were reviewed to evaluate their roentgenographic response (table 10). The interpretation was not carried beyond the last film prior to surgery if the patient had surgery performed. The observation time was essentially the same as reported above in the analysis of sputum "conversion" after chemotherapy. There were 13 patients (23.2 per cent) whose roentgenograms were interpreted as showing no change, 18 (32 per cent) whose disease showed slight improvement, 4 (7 per cent) whose lesions showed moderate improvement, and 7 (12.5 per cent) whose disease showed marked improvement. Fourteen patients (25 per cent) were classified as worse, and 7 of these had died. Again an attempt was made to correlate roentgenographic response with drug regimen, but the findings were inconclusive.

Surgical procedures: The indications for chest surgery in this group of patients, as well as the criteria for selection of type and extent of surgery, were the same as for patients with disease caused by typical organisms. Thirteen patients had chest surgery performed (table 11); twelve of these were "positive" at the time of surgery. One patient had "converted" his sputum prior to surgery, but a segmental resection was done for removal of fibrocaseous calcific residual. One patient had two procedures; first, a definitive type of thoracoplasty and, later,

TABLE 11

SURGICAL PROCEDURES ON 13 PATIENTS

	Number	Failures	Successes
A. Thoracoplasty			
2-stage	4	2	2
Preresection	1	0	1
Plombage	1	1	0
B. Resections			
Pneumonectomy	1	0	1
Segmental	3	1	2
Lobectomies	3	2	1
C. Monaldi procedure	1	0	1
Totals	14	6	8

Note: One patient had two procedures, a definitive thoracoplasty and later a lobectomy.

a lobectomy, both of which failed to convert his sputum. The other 12 patients had only one definitive procedure each. There were four two-stage thoracoplasties; two were successful and two were failures. In one of the failures late sputum "conversion" occurred after the patient had left the hospital. One patient "converted" his sputum following a two-rib preresection thoracoplasty. Further surgery was deferred in this case because of an intercurrent hepatitis, but the patient's sputum remained permanently noninfectious and no further surgery was done. One patient had a plombage thoracoplasty and is still discharging acid-fast bacilli. In the resection group, one patient had a pneumonectomy which was a success. There were three segmental resections. One was a failure and two were successful, but in one of the patients the sputum had become noninfectious prior to surgery. There were three lobectomies. Two were failures and one was successful. One Monaldi procedure was successful in "converting" the patient's sputum. One postoperative death occurred in the group. This patient had a lobectomy and died from postoperative cardiac failure. The present status of the 13 patients is as follows: one is now dead, 8 are now sputum "negative," and 4 continue to discharge acid-fast bacilli.

Pathologic studies: Studies of the resected surgical specimens from 7 patients revealed gross and histopathologic findings which were identical with the pathologic manifestations usually associated with infection due to typical *M. tuberculosis* (table 12). Such findings as tuberculous bronchopneumonia, tuberculous endobronchitis, draining cavities, and tuberculomata were noted (figures 1, 2, 3*A*, *B*, and *C*). All of the specimens had pathologic lesions which were positive for acid-fast bacilli on direct smear, and the cultures of the surgical specimens were positive for atypical bacilli in 6 of the 7 cases. One specimen was "negative" on culture. Six patients had been discharging acid-fast bacilli in the sputum at the time of surgery, but one patient had "converted" on drug therapy prior to surgery. The surgical specimen of this patient was positive on microscopy, but the culture was negative for atypical bacilli.

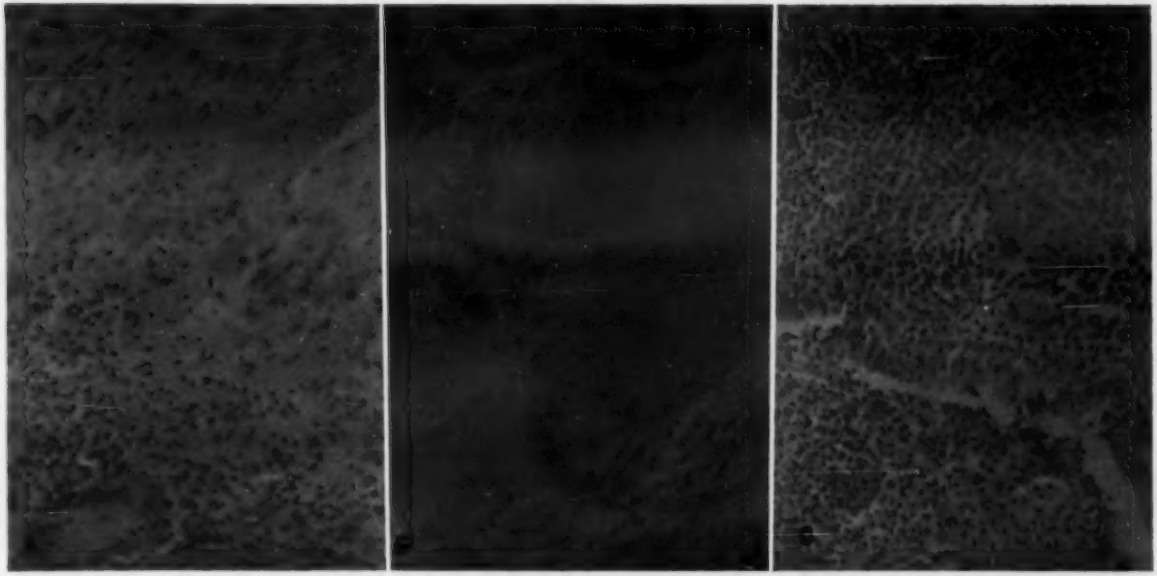
TABLE 12

PATHOLOGY TABLE OF RESECTED SPECIMENS

Patients	Race	Sex	Age	Specimen	Diagnosis	Laboratory Work Microscopy and Culture	Guinea Pig	Drug Susceptibility
AW	C	M	45	Left lung	Residual fibrocaseous tuberculosis	Microscopy of lesions positive; culture positive, atypical, six weeks	Postive, 5 mg. pooled suspension of pulmonary tissue	Totally resistant to isoniazid, partially resistant to streptomycin, no isoniazid medication
CP	W	M	50	Apical Subsegment LUL	Large, draining tuberculous cavity and fibrocaseous tuberculosis; tuberculous endobronchitis	Microscopy of lesion and bronchus, positive; culture positive, atypical (six weeks)	Negative	Partially resistant to isoniazid and streptomycin
JBE	W	M	35	Posterior Subsegment LUL	Active bronchopneumonic fibrocaseous tuberculosis and filled cavity; tuberculous endobronchitis	Microscopy of lesion and bronchus, positive; culture positive, atypical (six weeks)	Questionable OT after 8 weeks. Positive, common iliac node	Totally resistant to isoniazid; partially resistant to streptomycin
AP	W	M	45	RUL and ML Wedge	Diffuse nodular fibrocaseous tuberculosis with one small cavity, posterior segment	Microscopy of lesions positive; cultures positive, atypical (six weeks)	Negative, abscess at site of injection from pooled culture	Totally resistant to isoniazid; partially resistant to streptomycin
RC	W	M	44	LUL	Residual fibrocaseous calcific tuberculosis	Microscopy of lesions positive; cultures negative after seven months	Negative	Totally resistant to isoniazid; partially resistant to streptomycin
AC	W	M	41	RUL	Residual fibrocaseous tuberculosis with 3 cavities; tuberculous endobronchitis	Microscopy of lesions and bronchus positive; cultures positive atypical (six weeks)	Negative	Totally resistant to isoniazid; partially resistant to streptomycin
GD	W	M	43	RUL	Residual fibrocaseous disease with large apical and posterior segmental cavity; tuberculous endobronchitis	Microscopy: cavity and bronchus positive; culture positive, atypical (eight, weeks)	Negative, suspicious common iliac node and positive on reculture	Totally resistant to isoniazid; partially resistant to streptomycin

FIG. 1. *Case 2* (table 12). Apical segment showing large openly drained tuberculous cavity.

FIG. 2. *Case 4* (table 12). Segment of right upper lobe and middle lobe wedge, showing scattered fibrocaseous lesions.



Case 2 (table 12)

FIG. 3*A*. Photomicrograph showing cavity wall with Langhans' giant cell. *B*. Photomicrograph showing portion of tuberculoma. *C*. Photomicrograph showing tuberculous endobronchitis.

TABLE 13

PRESENT STATUS

		At Discharge	
		Positive for Acid-Fast Bacilli	Negative for Acid-Fast Bacilli
Dead*	10		
Hospital 3		3	0
Home 7		7	0
Hospitalized	16	15	1
At home	43	20	23 †
Totals	69	45	24 (34.7%)

* One additional death after study.

† 17 after chemotherapy; 7 after surgery.

Certified Cause of Death

Tuberculosis	7
Myocardial infarction	1
Postoperative cardiac failure	1
Unknown	1
Total	10

Present status: At the time of completion of the study, 10 patients were known to have died. Three deaths occurred in the hospital and 7 at home (table 13). All patients who died were discharging acid-fast bacilli in the sputum at the time of death if they were in the hospital, and had been "positive" on discharge if they later died at home. Certified causes of death revealed that the cause of death was given as tuberculosis in 7 cases; coronary occlusion in one case; cardiac failure in one case; and in one case, cause unknown. There has been one additional death from tuberculosis since the end of the study. Still hospitalized at the end of the study were 16 patients, 15 of whom continued to have acid-fast bacilli in the sputum. Forty-three patients were discharged by regular or unsanctioned discharge, 37 of whom were traceable and alive, and 7 were lost to observation but were presumed to be alive. At the end of the study 24 (34.7 per cent) of the total number of 69 patients had "converted" their sputum for three months or longer prior to discharge. There were 17 "conversions" on chemotherapy and 7 "conversions" after surgery (table 13). Follow-up sputum studies were attempted on all discharged patients but were inadequate for purposes of current evaluation.

Epidemiology: After reviewing the family history and age incidence of these patients on admission to the hospital, it was noted that 80 per cent had a family history negative for tuberculosis. In the 63 married patients, there was not a single instance of a history of tuberculosis in the spouse. The incidence of patients less than forty years old on admission was low. These and other observations caused the question to be raised as to the degree of infectiousness of these patients with active pulmonary disease simulating tuberculosis but associated with the presence of atypical acid-fast bacilli in the sputum.

TABLE 14

TUBERCULIN REACTION TO 0.01 MG. OF OT AMONG HOUSEHOLD CONTACTS OF 51 PATIENTS WITH SPUTUM POSITIVE FOR ATYPICAL MYCOBACTERIA

Number of Contacts Skin Tested	Number Positive	Per Cent	Number Negative	Per Cent	Total	Per Cent
122	35	28.6	87	71.3	122	100

Beeuwkes *et al.* (9) tuberculin reaction in a survey among household contacts of tuberculous patients with sputum positive for *M. tuberculosis*:

First Tuberculin Number Tested	Number Positive	Per Cent	Number Negative	Per Cent	Total	Per Cent
743	590	79.4	153	20.6	743	100

In search for data pertaining to this question, the existing information on the incidence of infection in family contacts, as judged by the tuberculin reaction and roentgenographic findings in these contacts, was obtained from the State Director of Tuberculosis Control and local health departments in Georgia. Fifty-one of the 69 patients had 243 known family household contacts. For various reasons, information on the contacts of the remaining 18 patients has not become available for study. Of the 243 known contacts, there were 122 (50 per cent) on whom tuberculin skin tests had been done. Positive skin tests in persons of all ages were recorded in only 29 per cent of these (table 14). A comparison of this figure was made with the observations of Beeuwkes, Hahn, and Putnam (9), who made a large survey of contacts of known "sputum-positive" patients. The comparison showed the percentage of their reactors was almost three times greater than in the group of contacts in the present study.

Two hundred and ten of the 243 known household contacts had had chest roentgenographic examinations. Only 7 (3.3 per cent) had roentgenographic evidence suggestive of tuberculosis, and 4 (1.9 per cent) contact films were read as suspicious (table 15). Thus only 11 (5.2 per cent) of the total of 210 contacts with chest roentgenograms were considered to show features suggestive of tuberculosis. This finding was compared with the observation of Beeuwkes and associates in a roentgenographic survey of contacts of known "sputum-positive" patients of all ages in the study mentioned above. Their study showed 44 per cent of contacts had chest roentgenograms showing calcified nodular or latent and manifest lesions suggestive of tuberculosis.

In search for more information on this question, a study was made of skin tests and roentgenograms of the household contacts and the family history of the 7 surgically resected patients (table 16). As stated before, the resected specimens of all 7 patients showed histopathologic findings indistinguishable from tuberculosis; and all lesions were positive on direct smear, and 6 were positive for atypical acid-fast bacilli on culture. These 7 patients had 26 known household contacts. Tuberculin tests had been performed on 23 of these, and only 4 (15.4

TABLE 15

RESULTS OF ROENTGENOGRAPHIC EXAMINATION AMONG CONTACTS OF 51 PATIENTS WITH SPUTUM POSITIVE FOR ATYPICAL MYCOBACTERIA

Number of Contacts Examined Roentgenographically	Number Positive	Per Cent	Number Negative	Per Cent	Total	Per Cent
210	7	3.3	199	94.7	206	98*

Beeuwkes *et al.* results of roentgenographic examinations in the same survey mentioned in table No. 14:

Number of Contacts Examined Roentgenographically	Number Positive	Per Cent	Number Negative	Per Cent	Total	Per Cent
987	439	44	548	56	987	100

* Four cases (1.9 per cent) were read as suspicious for a tuberculous lesion.

TABLE 16

EPIDEMIOLOGIC FINDINGS AMONG THE PATIENTS WHO RECEIVED RESECTIONAL SURGERY

Number of Patients-7	Number of Contacts Examined	Number Skin Tested	Skin Test		Number Filmed	Roentgenogram		Family History	
			Positive	Negative		Positive	Negative	Positive	Negative
Patient No. 1	0	0			0				1
Patient No. 2	6	5	2	3	6	0	6	?	
Patient No. 3	2	2	1	1	2	0	2	0	1
Patient No. 4	8	8	0	8	8	0	8	1	
Patient No. 5	5	5	1	4	5	Suspicious 1	4	0	1
Patient No. 6	3	3	0	3	3	0	3		1
Patient No. 7	2				2	1	1		1
Totals	26	23	4	19	26	2	24	2	5

per cent) were "positive." Chest roentgenograms had been made on all 26 contacts, and only 2 (7.2 per cent) had evidence suggestive of tuberculosis. The family history was positive for a history of tuberculosis in 2 of the 7 patients.

A very interesting situation which reveals many of the features of patients with atypical organisms was presented by one patient (*Patient No. 4*, table 16). This patient had 8 household contacts. These contacts had lived continuously with him, and, according to the history, he had had a cough "for many years." None of the 8 members of his family reacted to tuberculin and all had had roentgenograms interpreted as normal. The patient's sputum was repeatedly positive on culture for atypical bacilli. He failed to "convert" his sputum on

TABLE 17

SOME COMPARISON BETWEEN PATIENTS WITH ATYPICAL MYCOBACTERIUM AND THOSE WITH TYPICAL MYCOBACTERIUM TUBERCULOSIS

	69 Atypical		138 Typical	
	Number	Per Cent	Number	Per Cent
Family history positive for tuberculosis	12	17.4	41	29.7
Family history questionable	2	2.8	9	6.5
Family history negative	55	79.7	88	63.7
Marital history (63 married) positive	0	0	10	8.0*
Marital history negative	63	100.0	113	91.0
Tuberculin positive to 0.01 mg	40	57.9	132	95.6
Related complications:				
Intrapulmonary (1 or more per patient)	33		33	
Extrapulmonary (1 or more per patient)	0		13	
Patients admitted less than 30 years of age	2	2.8	47	34.0
Acutely ill on admission	1†	1.4	26	18.8
Died of pulmonary (atypical) tuberculosis	7	10.0	12	8.7
Died of nontuberculous disease	2	2.8	0	0
Died, cause unknown	1	1.4		

* 123 married.

† Due to status asthmaticus; no infiltration in lungs.

chemotherapy. He had a therapeutically unsuccessful thoracoplasty and subsequently an unsuccessful lobectomy. His resected specimen showed pathologic changes indistinguishable from tuberculosis. Material from the resected specimen was positive on microscopy and culture for atypical bacilli. The patient continued to discharge acid-fast bacilli in the sputum after surgery. Originally chronically ill on admission, the patient was in good condition at the time of his unsanctioned discharge. He firmly believed that "his atypical infection was not catching." He had no answer when asked how he had acquired the infection.

Comparison with typical M. tuberculosis: Some of the data accumulated and already presented under Observations was tabulated, and a comparative study was made with similar observations obtained from a study of the records of 138 white and Negro patients whose sputum contained typical tubercle bacilli. The latter cases were consecutive admissions, and the number selected—twice that of the atypicals—was purely arbitrary (table 17).

DISCUSSION

Many of the various observations made on the characteristics of these patients either need no discussion or their significance is not clear. Certain other of the observations merit consideration.

In evaluation of the racial incidence of these patients the number of Negro patients was very few. If this observation continues to be true in a large and significant series, then it might suggest that the Negro possesses some—or possibly a high—degree of immunity to these atypical bacilli. In reviewing the age incidence, there were only 2 patients less than thirty and only 17.3 per cent less

than forty years of age. This observation might suggest that a long period of contact and perhaps large repeated doses of these organisms over a long period of time are required to produce clinical disease. Other workers (5) studying atypical acid-fast bacilli, although possibly different from the present strains, have demonstrated that some guinea pigs apparently resistant to a standard inoculum were susceptible when given high-dosage inoculations.

The study of the past histories of the patients revealed a somewhat higher incidence of respiratory diseases than was expected. There were 14 patients with a previous diagnosis of tuberculosis made by symptoms, roentgenograms, or bacteriologic studies, and 11 of these had been known to have had previously acid-fast bacilli in the sputum on microscopy. This observation might indicate that in the years prior to the widespread use of cultural proof of bacterial identity these 14 patients and perhaps others have masqueraded or been carried as bona fide cases of tuberculosis. It certainly cannot be said about these 14 cases that their lesions were not caused by, or at least associated with, atypical acid-fast bacilli at the time of their first diagnosis.

The number of tuberculin reactors to first-strength tuberculin (0.01 mg.) was low, but the implication of this finding was not clear. The finding that not a single patient had a tuberculous extrapulmonary complication was surprising and might indicate that these organisms have a low metastatic potential. It was also wondered if this observation might not be correlated with the usual finding in the guinea pig of only localized limited disease if the pig did show any evidence of infection.

Regarding the cultural characteristics of these organisms, it is apparent that no common agreement has been reached by bacteriologists over the country on the classification of acid-fast bacilli labeled as atypical on culture. Reports from widely scattered sections of the country and even from local areas reveal that there are differences in cultural growth and other characteristics. Also reported has been variation of strains on subculture. Cultures from 64 of the patients in the present series were smooth, buff-colored, moist, slow-growing strains, practically avirulent for the guinea pig. The other five strains in the series were comprised of three chromogenic and two rough, rapidly growing types. It is now realized that the patients with the latter five strains should have had separate and distinct correlation with clinical, pathologic, and epidemiologic aspects of their concomitant pulmonary disease.

The finding that a considerable number of these patient-strains were partially or completely resistant to isoniazid prior to chemotherapy was an interesting one. A late study of 22 patients with atypical cultures who were not included in the original study revealed this observation to be more pronounced. It was definitely thought that these observations did not mean that these patients had acquired their infection from another person who had been treated with isoniazid. On the contrary, many of the patients in the original group were admitted before 1953 when isoniazid was first used in Georgia in any appreciable amount. Since 1953 isoniazid has been in widespread use, but the low incidence of family history and the low incidental contact history of these patients with known cases of tuber-

culosis would make one strongly doubt that these atypical organisms might be isoniazid-resistant mutants of *M. tuberculosis* acquired in such fashion.

The animal virulence studies would indicate that these organisms are for all practical purposes avirulent for the guinea pig and cause no infection in rabbits or chickens. The finding of progressive disease in the guinea pigs of only 2 patients and only localized limited evidence of disease in those of 21 patients, most of which were challenged with high inocula, bears this out. It was wondered if perhaps other routes of inoculation should not be attempted, particularly inhalation, in view of the already expressed thought that the metastatic potential of these organisms may be low.

The rate of sputum "conversion" on antimicrobial therapy was disappointingly low; only 30.3 per cent of 56 patients attained sputum cultures negative for acid-fast bacilli for three months or more. However, no patient "converted" his sputum who did not receive chemotherapy. These observations suggest that the infection caused by atypical organisms is a stubborn one to treat. It is conceivable that, in a larger series of cases with less pulmonary destruction than was encountered in this group, the results might be more favorable. The roentgenographic response to chemotherapy was also poor; only 11 of the 56 patients experienced moderate or marked improvement. With further reference to poor response to drugs, there was one dramatic case in which there was a small noncavitary exudative area in the left apex. After six months of chemotherapy this lesion sloughed to form a 3 cm. cavity. Surgical excision was done, and the pathologic findings showed lesions indistinguishable from tuberculosis. Prior to surgery the patient's sputum was persistently positive for only atypical bacilli which were avirulent for the guinea pig. The surgical specimen contained only atypical organisms which likewise were avirulent for the guinea pig. The over-all poor response shown by these patients has caused quite a problem in regard to prolonged hospitalization and brings up the question of public health implications if they are discharged when they have reached maximal hospital benefit while still discharging acid-fast bacilli in the sputum.

The 13 patients who had chest surgery performed had varied surgical procedures. This group is obviously not large enough to evaluate the type of surgery most advisable. The results of surgery could not be considered satisfactory as only 7 of 12 infectious patients (58.4 per cent) "converted" their sputum following surgery. However, the results with surgery were better than with chemotherapy alone. Because of this, it is believed that surgical intervention is warranted whenever possible, particularly when the disease is localized. It is also believed at this hospital that early surgery is indicated; that complete extirpative resection be done if possible; that resection is preferable to thoracoplasty; and that probably lobectomy is preferable to segmental resection. Problems arising with individual patients in the present series have given rise to these opinions.

The pathologic studies deserve comment. The findings on resected surgical specimens were identical in all cases with those due to typical *M. tuberculosis*. Lesions from the resected specimens were positive on microscopy, and in 6 of 7 cases the resected specimens were positive on culture for atypical organisms. The

pathologic findings would seem to substantiate the belief of the present writers that the atypical organisms isolated from patients in this hospital were capable of causing and did cause pulmonary disease indistinguishable from that caused by *M. tuberculosis* as shown by gross and microscopic examination of diseased tissue. From the resected specimens, certainly no evidence was found to suggest that these organisms were not the cause of the pulmonary disease manifested. An analysis of the present status of these patients revealed that only 34.7 per cent of the 69 patients had "converted" their sputum for three months or longer at the end of the study by all methods of treatment, again showing this disease a stubborn one to treat. The mortality data revealed that 10 patients (14.5 per cent) have died. One additional patient has died since the end of the study. All patients who died had not "converted" their sputum. There were 7 deaths, with tuberculosis given as the cause of death; 3 of the patients died in the hospital. Unfortunately there were no autopsies performed in any of the fatal cases. The implication of this mortality rate is that the atypical organisms isolated from patients in this hospital potentially may cause progressive pulmonary disease and death.

The epidemiologic findings were among the most interesting in the study. The data accumulated on family history, history of tuberculosis in the marital partner, tuberculin skin tests and roentgenograms of household contacts, all would seem to suggest that disease due to these atypical organisms is less communicable than typical *M. tuberculosis*. For example, in surveys made by Hahn (8), Barclay (10), Haywood, Morris, and Wilson (11), it was shown that the tuberculin reactors among contacts of a large number of tuberculous patients ranged from 57 per cent in those less than nineteen (10) to 92 per cent in all ages (11); that the history of tuberculous husbands (8) showed the wife to have active lesions in 7.9 per cent and asymptomatic lesions in 11.8 per cent; that the history of tuberculous wives showed the husband to have active lesions in 5.3 per cent and asymptomatic lesions in 18.6 per cent; that the children of tuberculous parents showed clinical tuberculosis in approximately 5 per cent and asymptomatic tuberculosis in approximately 6 per cent. Haywood and associates (11) found manifest lesions in 38 per cent of 604 contacts and that 49 (36 per cent) of 156 families of tuberculous patients were positive for tuberculosis. Barclay (10) found "the incidence of tuberculosis in the contacts of bacillary primaries to be 24.6 per cent." These findings would seem to suggest that the rate of infection and disease found in the foregoing surveys are higher than in the group of the present study. It is true that the living standards have considerably improved since the above surveys were made, but it is doubtful that this would account for the difference in the rate of infection and disease between the contacts from the reported studies and the contacts of the patients in the present study.

The public health implications of these findings are not entirely clear. The series is quite small. These patients do create a problem of long-time management when they fail to "convert" their sputum. Many of them eventually leave the hospital by unsanctioned discharge or are considered for maximal hospital benefit discharges despite the continued presence of acid-fast bacilli in the sputum. Their

disease appears to be of low-grade communicability, but to just what degree remains to be seen.

Many vexing problems exist with respect to the pulmonary disease caused by these organisms. More effort is needed on bacteriologic classification of all potentially pathogenic atypical acid-fast bacilli. The source and mode of infection, presumed to be exactly the same as for typical *M. tuberculosis*, needs clarification. The role of previous nontuberculous lung disease in possibly preparing the ground for infection with these organisms needs more study. An attempt needs to be made to recover these organisms from surgical specimens of patients having nontuberculous chest surgery, such as for bronchiectasis, carcinoma, or lung abscess. There needs to be an intensive study of the sputum of these patients and of their resected specimens for other possible pathogens. More knowledge of communicability is desirable for practical purposes of management. More work needs to be done on duration of exposure of contacts, in the culturing of repeated sputum specimens of all contacts, including the primary case when the family history is positive for tuberculosis.

Pending the acquisition of data on these and many other such questions, it is believed that a practical plan of management can be stated, even though the present studies indicate a lessened degree of infectiousness associated with these atypical organisms. Except that the need for surgery seems more pronounced in these cases, it is the belief of the writers that patients with pulmonary disease associated with atypical bacilli on culture should be treated exactly like patients infected with *M. tuberculosis*. This appears to be especially indicated with respect to treatment and should also apply to public health precautions until more information is available.

Summary

A limited study was made on the clinical, pathologic, and epidemiologic features of 69 patients with pulmonary disease associated with atypical acid-fast bacilli in their sputum. These patients were admitted to the hospital over a five and a half year period and comprise about 1 per cent of the hospital admissions during these years.

The atypical organisms were of three different strains, but sixty-four of them were of the same type. These strains were for all practical purposes avirulent for the guinea pig, and most of them showed some degree of resistance to isoniazid prior to chemotherapy. From obtainable history there was no reason to believe that any patient had acquired his infection due to contact with an isoniazid-treated patient.

The incidence of infection with these organisms was low in the Negroes in the series. There were very few patients less than forty years of age. The incidence of previous respiratory diseases was high. The number of reactors to first-strength OT was less than was anticipated. There were no extrapulmonary complications.

The sputum "conversion" rate and the roentgenographic response to chemotherapy were poor. The results with surgery were fairly satisfactory in 13 patients. The resected specimens of 7 patients revealed pathologic findings indistinguish-

able from tuberculosis. All surgical specimens were positive for acid-fast bacilli on direct microscopy, and 6 of the 7 were positive on culture for atypical mycobacteria.

The sputum of only 24 patients (34.7 per cent) became noninfectious on culture for three months or longer by all methods of treatment. There have been 10 deaths in the series, 7 of which occurred from progressive pulmonary disease simulating tuberculosis.

Epidemiologic studies showed a low incidence of family history for tuberculosis. There were no two cases from the same family. Studies of tuberculin skin tests and roentgenograms of household contacts revealed the incidence of positive findings to be much less than was found in contacts of patients with *M. tuberculosis*.

Sumario

Limitado Estudio Clínico, Histopatológico y Epidemiológico de Enfermos con Lesiones Pulmonares Asociadas con Atípicos Bacilos Acidorresistentes en el Esputo

Este estudio limitado versó sobre las características clínicas, histopatológicas y epidemiológicas de 69 sujetos con enfermedad pulmonar asociada con bacilos ácidorresistentes en el esputo. Estos enfermos fueron coleccionados durante un período de cinco años y medio, comprendiendo aproximadamente uno por ciento de los ingresos en el hospital durante esos años.

Los gérmenes atípicos representaron 3 cepas distintas en los cultivos, pero 64 de ellos eran del mismo tipo. Esas cepas eran para todo fin práctico avirulentas para el cobayo, mostrando la mayoría alguna resistencia a la isoniacida antes de la quimioterapia. No había motivo alguno para creer que ninguna de esas cepas procediera de enfermos tratados con isoniacida.

La incidencia de infección con esos microbios fué baja en la raza negra. Fueron poquísimos los enfermos de menos de cuarenta años de edad. La incidencia de previas enfermedades de aparato respiratorio fué alta. El número de reactores a la primera dilución de TA fué menor de lo esperado. No hubo complicaciones extrapulmonares.

El índice de viraje del esputo y la respuesta radiográfica a la quimioterapia fueron malos. Los resultados obtenidos con la cirugía fueron medianos en 13 enfermos. Los ejemplares resecados de 7 enfermos revelaron una histopatología indistinguible de la tuberculosa. Todos los ejemplares quirúrgicos resultaron positivos a la microscopía directa, y 6 de los 7 fueron positivos para bacilos atípicos en los cultivos.

Sólo en 24 enfermos (34,7 por ciento) se volvió el esputo no infeccioso en los cultivos durante tres meses o más con ninguno de los métodos de tratamiento. Ha habido 13 muertes en la serie, debiéndose 7 de ellas a enfermedad pulmonar evolutiva simulando tuberculosis.

Los estudios epidemiológicos revelaron una incidencia baja de antecedentes familiares de tuberculosis. No hubo dos casos procedentes de la misma familia y la incidencia de tuberculosis en los esposos fué nula. Estudios de las cutirreaciones a la tuberculina y radiografías de los contactos caseros revelaron que la incidencia de hallazgos positivos era mucho menor que la observada en los contactos de enfermos con *M. tuberculosis*.

Resume

Etude clinique, pathologique et epidémiologique limitée de malades porteurs de lésions pulmonaires associées à la présence de bacilles acido-résistants atypiques

Une étude limitée a été effectuée chez 69 malades sur les aspects cliniques, pathologiques et épidémiologiques d'une affection pulmonaire avec bacilles acido-résistants atypiques dans l'expectoration. Ces cas ont été réunis durant une période de 5 ans et demi et comprennent environ 1% du total des admissions à l'hôpital durant ce temps.

Les germes atypiques ont donné en culture 3 souches différentes, mais 64 d'entre eux appartenaient au même type. Du point de vue pratique, ces souches étaient avirulentes pour le cobaye, et la plupart d'entre elles présentaient un certain degré de résistance à l'isoniazide avant la chimiothérapie. Il n'y avait pas lieu de penser que l'une ou l'autre de ces souches provenait de malades ayant été traités par l'isoniazide.

L'incidence de l'infection par ces germes était faible chez les nègres. Il y avait très peu de malades âgés de moins de 40 ans. La fréquence des affections respiratoires était élevée. Le nombre de cas réagissant à la dose minima d'OT était inférieur aux prévisions. Il n'y avait pas de complications extra-pulmonaires.

Les taux de négativation du crachat et des améliorations radiologiques à la suite de la chimiothérapie étaient faibles. Chez 13 malades les résultats obtenus par la chirurgie étaient moyens. Les pièces réséquées chez 7 malades révélaient des lésions indifférenciables de la tuberculose. Tous les tissus réséqués étaient positifs à l'examen microscopique direct, et 6 sur 7 ont donné des cultures positives pour les bacilles atypiques.

Chez 24 malades seulement (34,7 pour 100) la négativation du crachat en culture a été obtenue en trois mois ou un peu plus longtemps quelles que soient les méthodes de traitement. Il y a eu dans cette série dix décès, dont sept imputables à une affection pulmonaire évolutive ressemblant à la tuberculose.

Les recherches épidémiologiques indiquent la faible fréquence des antécédents tuberculeux familiaux; il n'y en avait pas deux cas dans une même famille et l'incidence de la tuberculose chez l'époux était nulle. L'étude des tests cutanés à la tuberculine et des radiographies de l'entourage du malade indique que l'incidence des résultats positifs est très inférieure à celle observée dans l'entourage des malades atteints de M. tuberculosis.

Addendum

Since this paper was completed and presented, correspondence has been received from Dr. Ernest H. Runyon, Director of Laboratory Services, Veterans Administration Hospital, Sunmount, New York (14). Dr. Runyon has had the opportunity to study a large number of the cultures from the present series as well as many atypical strains received from other widely scattered hospitals. Information received from him has lead to a much desired clarification of the bacteriologic classification of the atypical acid-fast organisms found associated with pulmonary disease in 64 of the patients in our series. Dr. Runyon has given the writers the following information. He states that the predominant type of bacillus in the present series, harbored by 64 of the 69 patients, belongs to a group of atypical acid-fast bacilli which have been designated as nonphotochromogens (15). According to Dr. Runyon, nonphotochromogenic atypical acid-fast bacilli indistinguishable from these strains have been isolated by very many widely scattered hospital laboratories and are available in the Sunmount collection.

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"OPEN HEALING" OF TUBERCULOUS CAVITIES[1, 2]

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INTRODUCTION

In recent literature (1–10) attention has been focused upon the phenomenon of "open healing" of tuberculous cavities.

Roentgenographically, these cavities are thin-walled, air-containing spaces, which may be solitary, joined, or in systems replacing areas of parenchyma. Grossly, they are lined by a smooth, grayish-lavender, glistening membrane which may be completely intact and healed. At times the membrane may show incomplete healing as evidenced by a break in continuity with millet-seed tubercles or tuberculous granulation tissue in an occasional crypt. In the experience of the present writers, epithelization has been extremely unusual and when observed was limited to the bronchocavitary margin.

REVIEW OF LITERATURE

Thompson (1) states: "While some argue that a cavity is not healed as long as the cavum remains open, it must be stressed that the disappearance of the tuberculous elements and replacement by fibrous tissue does constitute healing of the disease process per se." Johnson and Hewitt (2) refer to these same cavities as "cyst-like cavities." Sweaney and Seiler (3) state: "The walls of these cavities tend to become thin plates of connective tissue with no epithelium—having a smooth surface free of caseation and granulation tissue," and then refer to them as "bullae." Sweaney and Seiler also refer to one of their patients "having a large cavity measuring 10 cm. in diameter that apparently had completely sterilized. The wall of the cavity had a slick surface resembling that of any mucous membrane. There was no caseation remaining, nor any signs of inflammatory reaction. The exposed surfaces had no evidence of epithelium whatever. It was bare connective tissue that had developed a smooth surface." They use the term "bullous cavities." Bernou (10) states: "Following antimicrobial therapy a variety of cavities have been observed ranging from more or less voluminous pseudo-emphysematous bullae to sclerocystic cavities. Roentgenographically, the former have a very thin outline like an emphysematous bleb. Anatomically, the wall is flexible, thin, smooth, without caseation, and without macroscopic fibrous reaction. Sclerocystic cavities have a fibrous shell of varying thickness. The common trait is persistence of cavitation and inactivity due to healing of the walls."

Altmann and Mishima (11), in discussing the effects of isoniazid derivatives on the pathology of chronic pulmonary tuberculosis, list characteristics of the smooth-walled defects or cavities. One specimen containing large smooth-walled defects was described as being partly fibrotic with healed walls, partly actively tuberculous in spite of a gross appearance of healing, and the conclusion was that these were *probably healing tuberculous cavities*. Another patient had two cavities in the upper lobe with most of the wall appearing

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[1] From Battey State Hospital, Rome, Georgia.

[2] Presented before the Medical Session, as part of *Section 3A*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 22, 1956.

healed, but with a portion necrotic, and these were termed *partly healing bronchogenic cavities*. Another patient had interlocking large defects with fibrotic walls. There were residual, small active tuberculous foci in the wall and these were termed *almost completely healed cavities*. Poppe de Figueiredo and De Paola also describe the *complete healing* of cavitary walls following isoniazid therapy.

Caffey (13) states: "The frequency and importance of regional obstructive emphysema during acute respiratory infections have not generally been appreciated. Normal respiration is a check valvular phenomenon in all parts of the bronchial tree, since the bronchi dilate rapidly during inspiration and contract slowly during expiration. This normal check valvular effect is readily exaggerated by the local occlusive action of inflammatory exudate and mucosal swelling. As a result the regional alveoli become distended and in severe cases the alveolar walls may rupture. The coalescence of the dilated and ruptured alveoli may give rise to large cavities (pulmonary bullae) surrounded by peripheral strips of compression atelectasis (ring shadows). When the air escapes into the interstitial tissue of the lung it may localize in the sub-pleural connective tissue to form pulmonary blebs (interstitial emphysema). Pulmonary blebs and bullae cannot be satisfactorily differentiated roentgenographically."

Hinshaw and Garland (14) state: "Most air-containing cystic structures within the lung may be classified as either (*a*) alveolar cysts (blebs, bullae, and pneumatoceles), (*b*) bronchopulmonary cysts and (*c*) cystic bronchiectasis. Alveolar cysts are dilatations of alveoli; often many alveoli have fused by breakdown of the septa to contribute to the large air cysts. The lining of these spaces is similar to alveolar linings. Bronchopulmonary cysts are actual tumors, of congenital or developmental origin, and lined by bronchial epithelium. These are often solitary, relatively large and often contain fluid as well as air. They may be located within the lung or elsewhere, commonly in the mediastinum. Cystic bronchiectasis, like any bronchiectasis, is produced by dilatation of bronchi. The term cystic refers to the spherical shape of the dilatations, appearing vaguely like bunches of grapes when viewed in bronchograms. In other fields of medicine (dermatology) the words bleb and bulla are used synonymously with the popular word 'blister.' " Pulmonary bullae and blebs are ordinarily air-containing blisters. Some authors make no attempt to distinguish between bullae and blebs; others consider bullae to be larger than blebs, but otherwise identical. Others regard blebs as superficial, subpleural air blisters and consider bullae as deeper, intrapulmonary cystic lesions. Pneumatoceles are very large alveolar cysts containing air under positive pressure. Confusion is added when descriptive terms are used, including: honeycomb lung, cotton candy lung, and vanishing lung.

Caffey (13) states: "Congenital cystic disease is an extremely rare condition not having been found at necropsy in the Babies Hospital during a period of 50 years." Schenck quotes Lederer as "having failed to find a single instance of congenital cystic disease in 5,000 necropsies. It is now evident that many cases which have suggested congenital cysts, roentgenographically, were probably examples of a quiet emphysematous cavity caused by checked bronchial obstruction."

The above statements have been presented merely to show the considerable variations in thinking and in terminology that exist in regard to air-containing spaces in the lung. Quite a few of these air-containing spaces are being encountered in cases of pulmonary tuberculosis, treated with long-term drug therapy. The spaces are being termed cystic cavities, cystic lung, "open healing" of tuberculous cavities, bullous cavities, pseudo-emphysematous bullae, sclerocystic cavities, acquired tuberculous cysts, et cetera. The terminology is not as important

TABLE 1

SURGICAL MATERIAL STUDIED*

Pneumonectomies	73
Lobectomies	161
Segmental resections	160
Subsegmental resections	56
Wedge resections	33
Cavernectomies	3
Bronchogenic cystectomy	1
Total	487

* This table represents all consecutive surgical specimens submitted to the pathology department between January, 1952, and November, 1955.

TABLE 2

INCIDENCE OF "OPEN HEALING" AS SEEN IN VARIOUS TYPES OF RESECTED SPECIMENS

		Per Cent
Pneumonectomies	7 (of 73)	10
Lobectomies	14 (of 161)	9
Segmental resections	7 (of 160)	4
Cavernectomies	1 (of 3)	33
Wedge resections	1 (of 33)	3
Total	30 (of 487)	6

Open healing of tuberculous cavities has been observed in 6 per cent of the surgical material between January, 1952 and November, 1955.

as the understanding of the pathogenesis and pathology involved. As we gain more experience, a better perspective on therapy will be gained.

METHOD

Between January, 1952, and November, 1955, 487 consecutive pulmonary resection specimens were received in the pathology department for examination. In table 1 the type of resection is shown.

Gross, colored photography was first completed. During the gross pathologic examination, selected lesions were smeared under sterile conditions and material from these lesions was then submitted for further bacteriologic examination, including routine culture and animal inoculation—the latter if requested. The same lesions were then sectioned for microscopic examination, including from three to five sections per cavitary wall and at least one of the bronchocavitary margin. These were stained with hematoxylin-eosin. Ziehl-Neelsen stains were only used in one of the specimens in which fresh material for bacteriologic examination could not be obtained due to previous fixation.

OBSERVATIONS

"Open healing" of cavities was encountered in 30 specimens (6 per cent) of a total of 487 (table 2).

Fifteen of these were totally healed, and 15 were in the healing stage.

Examination of tables 3 and 4 will permit detailed evaluation of each case as

TABLE 3

HEALED CAVITIES

Initials and Case Number	Color Sex Age	Admission Date	Stage on Admission	Date of Last (+) Bacteriologic Examination	Drug Therapy and Duration Prior to Surgery	Surgery and Date	Pathology	Bacteriology of Resected Cavity
LM C-4042	N-M-32	4-30-51	FA*	4-13-53	Streptomycin, PAS, isoniazid, 15 months	Left apical posterior resection and superior division LLL wedge, 6-3-53	Cavity, apical, posterior segment, 1.3 cm. in diameter	(−)
JAC C-4004	N-M-23	3-26-51	FA	3-20-52	Streptomycin, PAS, isoniazid, 35 months	Left upper lobectomy and superior division, 2-3-54	Central UL cavity, 5 × 4 × 3 cm.	(−)
LB C-5035	N-F-32	2-9-53	FA	4-20-53	PAS and isoniazid, 19 months	Left pneumonectomy, 9-16-54	2 cavities, upper and lower lobes, 2–5 cm.	(−)
WHW C-5118	N-M-25	9-8-52 4-6-53	FA	2-18-54	Streptomycin, PAS, isoniazid, 24 months	Left pneumonectomy, 10-21-54	Communicating cavities, upper and lower lobes, 13 × 8 × 4 cm.	(−)
WLH A-21642	W-M-33	11-6-52	FA	1-8-54	Streptomycin, PAS, isoniazid, 23 months	LU lobectomy, 10-28-54	Cavities, upper lobe, 5–1 cm.	DS (−) Cult. (+)
AA C-5034	N-F-28	2-9-53	FA	2-25-53	Streptomycin, PAS, isoniazid, 20 months	RU lobectomy, 11-4-54	Central cavity, 10 × 5 × 3 cm.	(−)
MD C-5764	N-M-26	4-12-54	FA	4-13-54	Streptomycin and PAS, 13½ months	RU lobectomy and superior division RLL, 5-23-55	Central UL cavity, 10 × 6 × 3 cm.; S.D. 2.3 cm. in diameter	(−)
PP C-5361	N-M-29	Nontuberculous 4-14-52 9-7-53	FA	11-9-53	PAS and isoniazid, 21 months	RU lobectomy, 6-16-55	Cavity, anterior segment, 3 × 3 × 1 cm.	(−)

EB C-5607	N-M-37	1-18-54	FA	4-1-54	PAS and isoniazid, 17 months	Left pneumonectomy, 6-23-55	Cavity, apical posterior segment, 3 × 3 × 1 cm.	(−)
CC C-3365	N-M-25	9-5-49	FA	5-27-52	Streptomycin, PAS, isoniazid, 42 months	Left pneumonectomy, 9-8-53	Cavity, upper lobe, 11 × 6 × 3 cm.	(−)
SB C-3753	N-M-51	11-6-50	FA	5-9-52	Streptomycin, PAS, isoniazid, 18 months	RU lobectomy, 1-21-54	Cavity, upper lobe, 13 × 5 × 2 cm.	(−)
OMA C-5770	N-5770	4-19-54	FA	3-17-55	Streptomycin, PAS, isoniazid, 17 months	LU lobectomy and superior division LLL, 10-5-55	Cavity apical postero-anterior segment, 5 × 5 × 3 cm.	(−)
ARD C-5456	N-F-30	11-2-53	FA	11-4-53	Streptomycin, PAS, isoniazid, 17 months	LU lobectomy and superior division LLL, 8-9-55	Cavity, S.D., 1 × 1 × 0.3 cm.	(−)
LJ A-23431	W-M-29	10-28-54	FA	10-29-54	Streptomycin, PAS, isoniazid, 9½ months	Apical and posterior segmental resection, RUL, 8-10-55	Cavity, apical segment, 2 cm. in diameter	(−)
RM C-4946	N-F-23	12-8-52	FA	12-18-52	Streptomycin, PAS, isoniazid, 18 months	Left pneumonectomy, 6-10-54	4 cavities LL 5-6 cm. in diameter	(−)

* FA—Far advanced.

TABLE 4

HEALING CAVITIES

Initials and Case Number	Color Sex Age	Admission Date	Stage on Admission	Date of Last (+) Bacteriologic Examination	Drug Therapy and Duration Prior to Surgery	Surgery and Date	Pathology	Bacteriology of Resected Cavity
BSM A-23314	W-M-37	2-15-54 7-18-54 9-10-54	FA*	7-7-54	Streptomycin, PAS, isoniazid, 17 months	RU lobectomy, 7-18-55	Cavity, apical and posterior segment, 7 × 4 × 2 cm.	(−)
JMY C-5407	N-F-27	10-5-53	FA	4-15-54	PAS and isoniazid, 14 months	Apical, posterior and anterior segments, resection, left, 1-4-55	Cavity, apical and posterior segment, 7 × 5 × 2 cm.	(−)
ES C-5218	N-F-36	6-15-53	FA	10-15-53	Streptomycin and isoniazid, 22 months	RU lobectomy, 7-5-55	Central cavity, 3 × 3 × 2 cm.	No laboratory work done, fixed specimen
GHJ A-22283	W-M-36	8-10-53	FA	Partially resistant to both 5-5-55	Streptomycin, PAS, isoniazid, 21 months	RU lobectomy, 6-22-55	Cavity, apical and posterior segments, 3 × 2 × 2 cm.	(+) Direct smear and culture
GWD C-4889	N-M-30	10-8-51 10-27-52	FA	11-5-53	Streptomycin, PAS, isoniazid, 34 months	Left apical subsegmental resection, 5-2-55	Cavity, apical subsegments 2.3 cm. in diameter	(−)
MJP C-5095	N-F-15	3-21-53	FA	4-13-53	Streptomycin, PAS, isoniazid, 18 months	RU lobectomy; LL wedge resection, 8-26-54	Cavities, apical, posterior and anterior segments, 5-3 cm. in diameter	(−)

JW C-3864	N-M-43	1-4-51	FA	10-15-52	Streptomycin and PAS, 27 months	LU lobectomy, 4-23-53	Cavity, apical segment, 3 × 2 × 1 cm.	(−)
CM C-3531	N-F-20	6-27-49	MA†	2-9-51	Streptomycin, PAS, isoniazid, 27 months	RU lobectomy and S.D.,† 10-1-53	Cavity, posterior segment 0.4 cm. in diameter	(−)
JDC A-15598	W-M-36	7-26-44	FA	1-5-50	Streptomycin, PAS amithiozone 23 months	Left anterior segmental cavernectomy, 4-21-53	Cavity sac, 10 × 10 × 0.3 cm.	(−)
JRL A-20716	W-M-43	6-12-46 10-11-51	FA	8-25-52	Streptomycin, PAS, isoniazid, 15 months	Right apical posterior segments, anterior wedge, 10-22-52	Cavity, apical segment, 2 × 1 × 1 cm.	(−)
DT C-6102	N-M-19	10-11-54	FA	10-20-54	PAS and isoniazid, 12 months	Left apical subsegmental wedge, 10-12-55	Cavity, apical segment 0.3 cm. in diameter	(−)
MB C-5366	N-F-39	9-14-53	FA	12-2-53	Streptomycin, PAS, isoniazid, 22 months	Left pneumonectomy, 8-8-55	Cavity, apical posterior segment, 4×3× 2 cm.	(−)
BW C-5891	N F-40	6-21-54	FA	10-18-54	Streptomycin, PAS, isoniazid, 15 months	Right pneumonectomy, 11-14-55	Cavities, all 3 lobes, 6–10 cm.	(−)
DRQ A-22517	W-M-19	11-2-53	FA	9-3-54	Streptomycin, PAS, isoniazid, 24 months	Right apical segment, 10-25-55	Cavity, apical segment 1.5 cm. in diameter	(−)
RLS C-6323	N-F-36	11-27-50 11-28-52 8-24-53 2-21-55	FA	9-22-55	Streptomycin, PAS, isoniazid, 18½ months	RU lobectomy and superior division cavernectomy, R.L.L., 11-28-55	Cavities, apical segment, superior division RLL, 0.6 diam. to 4 × 2 × 2 cm.	(−)

* Far advanced.

† Moderately advanced.

to color, sex, age, admission date, admission diagnosis, date of last sputum culture positive for tubercle bacilli, drug regimen employed and duration thereof prior to surgery, the type of surgery and date thereof, pathologic classification to determine whether the cavities were healed or healing, and the results of the bacteriologic studies on the cavities.

Grossly, the healed cavities were found to be lined by a smooth, grayish-lavender to grayish-pink, glistening membrane. If located peripherally, the membrane might have a portion of tissue paper thickness, whereas more centrally located parts of these membranous walls might consist of fibrous tissue up to 0.2 cm. in thickness. In a few instances, slender, traversing trabeculations have been observed. A communicating, openly draining bronchus could *always* be demonstrated. Occasionally, a deep-seated caseous focus was observed in the adjacent parenchyma. The healing cavities exhibited rare, millet-seed-sized tubercles in the otherwise smooth, glistening, membranous tissue, or an occasional small, circumscribed focus of pinkish-gray granulation tissue. On microscopic examination, these foci showed caseating and noncaseating tubercles with giant cells, mostly of the Langhans' variety, and from these areas there was a direct transition to the already healed part of the cavity wall. The latter exhibited a few to several layers of hyalinizing, lamellarly arranged connective tissue, rather poor in vascular supply, showing scant scattered compressed capillaries and occasional small pericapillary lymphocytic infiltrates. The innermost lining revealed compressed elongated cells not resembling epithelium. Epithelium extending into the cavity for a very short distance beyond the bronchocavitary junction has been observed in one case. A moderately frequent finding in the healing cavities was the presence of scattered calcium deposits in the different layers of the wall. All of the cavities, healed and healing, were well circumscribed and marginated from the adjacent parenchyma. Anthracotic pigmentation in the cavitary wall was rare (tables 5–8).

"Open healing" occurred three times more frequently in Negroes. It was observed in the healing process of far advanced cases almost exclusively. It was not observed in a white female in this study. It was observed to occur most frequently when a triple-drug regimen of isoniazid-streptomycin-PAS had been used (tables 9 and 10).

TABLE 5

ADMISSION AGE DISTRIBUTION OF PATIENTS EXHIBITING OPEN HEALING OF CAVITIES

15–19	4
20–24	3
25–29	7
30–34	5
35–39	7
40–44	3
45–49	
50–54	1
Total	30

TABLE 6

SEX AND COLOR DISTRIBUTION

White male	7
White female	0
Negro male	11
Negro female	12
Total	30

TABLE 7

STAGE OF DISEASE ON ADMISSION

Far advanced	29
Moderately advanced	1
Total	30

TABLE 8

DRUG REGIMENS EMPLOYED

Streptomycin, PAS, and isoniazid	21
Isoniazid and PAS	5
Streptomycin and PAS	2
Streptomycin, PAS, and amithiozone	1
Streptomycin and isoniazid	1
Total	30

TABLE 9

AVERAGE DURATION OF DRUG THERAPY PRIOR TO SURGERY

	Healed Cavities		Healing Cavities	
	Number of Cases	Number of Months	Number of Cases	Number of Months
Streptomycin, PAS, and isoniazid	11	22	10	21
Isoniazid and PAS	3	20	2	13
Streptomycin and PAS	1	13	1	27
Streptomycin, PAS, and amithiozone			1	23
Streptomycin and isoniazid			1	22

TABLE 10

DURATION OF SPUTUM STATUS AT TIME OF SURGERY

Negative for tubercle bacilli	30 cases
Time	15 months average per case

As may be seen in the tables, the patients with "open healing" had received long-term antituberculous drug therapy and had had no demonstrable tubercle bacilli in the sputum for an average of fifteen months prior to surgical excision (table 11).

TABLE 11

BACTERIOLOGY OF RESECTED CAVITIES

	Positive	Negative	Not Done
Healed	1*	14	
Healing	1	13	1

* Fibrocaseous nodule in parenchyma directly adjacent to cavity wall.

Of the cavities classified as being completely healed, 14 of the 15 revealed no tubercle bacilli on bacteriologic studies. A culture of one cavity was positive for tubercle bacilli and the microorganisms are believed to have arisen from a fibrocaseous nodule in the parenchyma directly adjacent to the cavity wall.

Of the cavities classified as healing, only one yielded tubercle bacilli on culture, 13 were negative, and one was not studied bacteriologically.

In all, negative bacteriologic studies were obtained on 27 of the 30 specimens of "open healing."

DISCUSSION

There is opposition to the term "open healing" of tuberculous cavities. Both from reviewing the literature and from talking with many phthisiologists, the present writers are certain that the phenomenon discussed in this paper is being observed throughout the country. It seems that everyone has his own "pet phrase" he likes to apply to this pathologic picture, but it is virtually certain that everyone is talking about the same pathologic picture.

Again reference is made to the work of Altmann and Mishima (11), who used the terms "probably healing tuberculous cavities," "partly healing bronchogenic cavities," "almost completely healed cavities." In one of their cases in which the smooth walls contained a very rare small active tuberculous focus, they too believed that this focus seemed to be the final proof that the peculiar cavitary system represented almost completely healed tuberculous cavities.

The writers' concept is that these cavities were lined by a necrotic membrane and were "dirty" prior to institution of drug therapy, particularly isoniazid. As time and therapy progressed, the cavities were cleansed, the dirty necrotic lining gradually being replaced by a smooth, shiny, clean membrane. In some cavities, which are herein termed healed cavities, this process has been complete. In other cavities this process has occurred but has not progressed to completion; in some, 99 per cent or more of the total surface is intact with the smooth clean membrane as previously described, but small foci of granulation, caseation, and even calcification are interspersed. This latter group is designated "open healing" cavities or incompletely healed cavities.

Several things are apparent. This pathologic picture has been seen in increasing frequency since the advent of isoniazid and its widespread use for long periods of time. In the present series of resections, 6 per cent of all specimens during the period of time considered contained one or more of these cavities. Thompson (1) included only cases of cavitary disease, and 9.6 per cent of his

240 cases showed changes typifying "open healing." There is no way roentgenographically that one can determine whether these cavities are in a healing or in a healed state. This can be done only after the pathologic specimen has been studied. These cavities originated in areas of the lung where tuberculosis and cavitation were clearly discernible and in patients who had had tubercle bacilli in the sputum. The "open healing" cavities occur three times more frequently in Negroes.

It should be stressed that these cavities are lined with fibrous tissue that is not similar to alveolar linings, nor are they lined by bronchial epithelium. The argument has been made that, if more than three sections had been taken through a cavity wall with multiple serial sections being the rule, at least half of the present cases would not be called healed cavities, as many more microscopic tubercles would have been found. The fact that tubercles may be seen in the fibrous wall of these cavities, however, does support the view that "open healing" does take place and further helps differentiate them from so-called blebs, bullae, and bronchial cysts. Whether the healing is complete is problematical, but the term "open healing" merely indicates that the cavity is open and that healing is taking place or has taken place.

The therapy in these cases is not always simple. Dugan and Hardy (15) state that the group of intrapulmonary space-occupying, air-containing lesions tend to cause disturbance in pulmonary function; to undergo suppuration, rupture, or hemorrhage; and that their surgical excision is mandatory. Head (16) states, in considering the treatment of cystic disease of the lung, that the selection of cases is difficult and extremely important. One must be sure that the vital capacity will be increased rather than decreased. Head states that lobectomy is rarely necessary. The extremely affected lung portions rarely comprise more than a few segments. It will be noted, in dealing with these openly healed or healing cavities of tuberculosis in this series, that 23 of the 30 patients who exhibited this phenomenon were treated by a lobectomy or a smaller resection. Seven of these patients, however, required a pneumonectomy because of the extent of disease.

The possibilities of rupture, suppuration, hemorrhage, and reactivation of tuberculosis are well recognized. Accordingly, it is believed that, when these openly healed or healing cavities are unilateral or bilateral, they shoud be excised if the patient is otherwise a fit candidate for surgery.

The writers are also seeing an increasing number of patients with extensive bilateral cavitation, with sputum consistently negative for tubercle bacilli under drug therapy. These patients, for one reason or another, are not candidates for surgery. Some of them have now been observed for a period of three years and are continuing to do well clinically and are remaining noninfectious.

Patients with the last-named syndrome pose quite a problem. They are not surgical candidates; they do not have infectious tuberculosis. They have open cavities and hence must be classified as active cases of pulmonary tuberculosis. It is believed that these patients should be followed carefully and such patients are being discharged by the writers from the hospital to return to their local communities to be followed and kept on drug therapy. There are few accumu-

lated data relative to what the ultimate course will be in these patients with persistent cavitation and noninfectious sputum.

The writers have observed a group of patients treated surgically and a group of patients who have been treated solely with chemotherapy. These are not comparable groups and this will not be a controlled study, as the patients have not been selected for surgery or for continued outpatient nonsurgical care by randomization. Nevertheless, it will be interesting to follow both groups of patients over a period of five or ten years and see what the comparison will show with respect to reactivation of disease, complications, morbidity, and deaths from pulmonary tuberculosis.

Summary

Thirty cases of openly healed and healing cavities in patients with pulmonary tuberculosis have been presented. Many pathologic terms are being used to describe what appears to be a common pathologic process. The choice of whether these cavitary lesions are to be treated surgically or observed on a long-term medical program must be individualized on the merits and status of each case.

Sumario

"Cicatrizacion Abierta" de las Cavernas Tuberculosas

Preséntanse 30 casos de cavernas cicatrizadas y en vías de cicatrización "abiertamente" en casos de tuberculosis pulmonar. Muchos son los términos patológicos usados para describir lo que parece ser un proceso patológico común. A base de los méritos y estado de cada caso hay que individualizar la eleccion entre si se tratarán quirúrgicamente estas lesiones o se mantendrán en observación con un plan médico a largo plazo.

Resume

Cavernes tuberculeuses épithélialisées avec crachats abacillaires ("Open Healing")

Présentation de trente cas de tuberculose pulmonaire avec excavations nettement épithélialisées ou en cours d'épithélialisation. De nombreux termes de pathologie sont employés pour décrire ce qui paraît être un processus pathologique commun. La décision de traiter chirurgicalement ces lésions cavitaires ou de les maintenir en observation durant un programme à long terme demande une étude particulière des avantages présentés et de l'état du malade.

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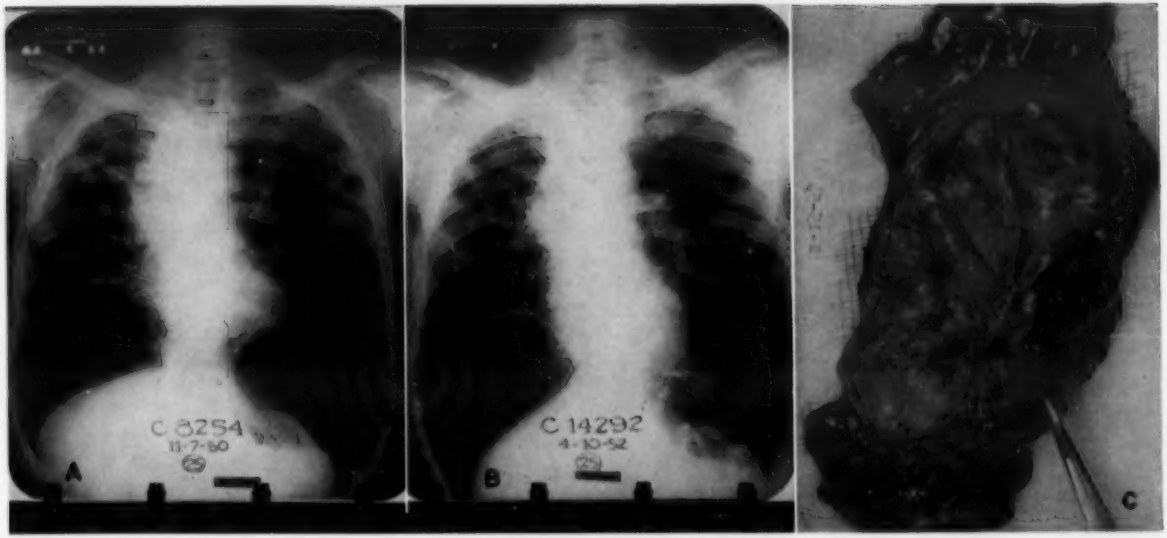
Figures 1–6 follow

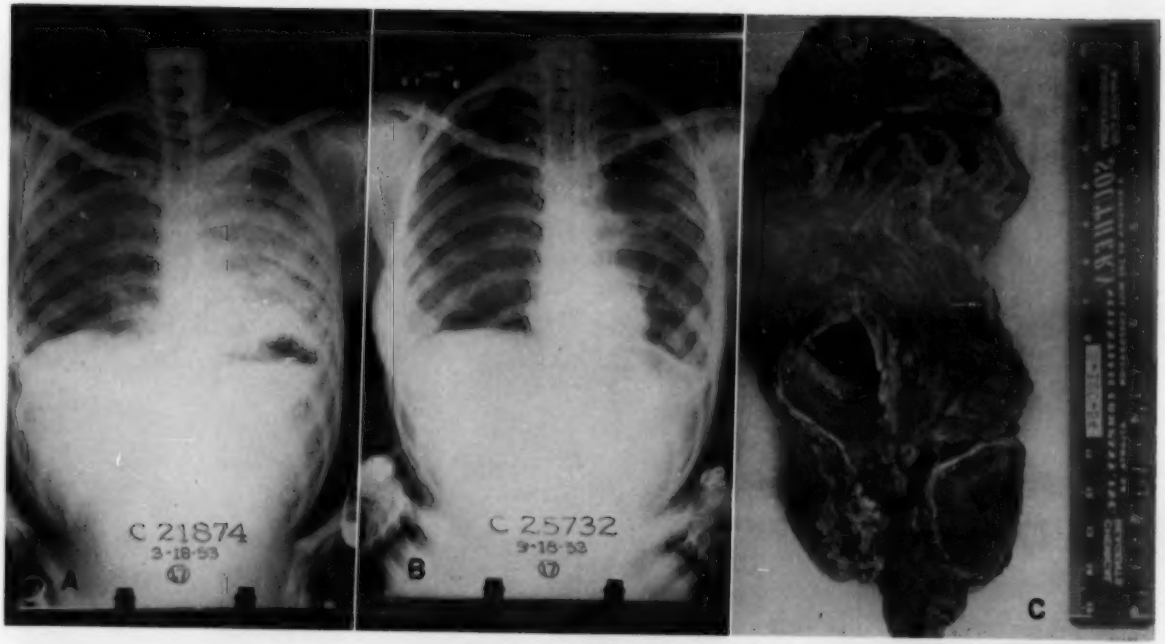


Case 11 (table 3)

FIG. 1*A*. Chest roentgenogram showing the disease on the patient's admission to the hospital, with right upper lobe cavity.

B. Chest roentgenogram showing the openly healed cavity in the right upper lobe.

C. Gross specimen showing the entrance of the bronchus, which is opened and just in front of the hemostat, into this cavity, which is completely healed with an intact, shiny, glistening membrane throughout.

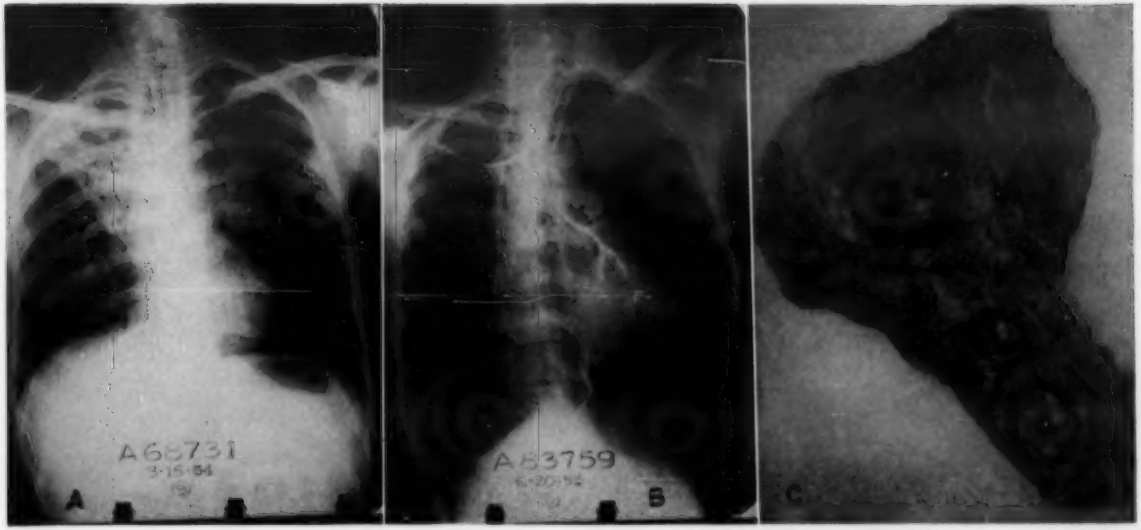


Case 15 (table 3)

FIG. 2*A*. Chest roentgenogram showing extensive bilateral disease on the patient's admission to the hospital.

B. Chest roentgenogram showing a destroyed lung with multiple cavities after six months of drug therapy.

C. Gross specimen showing the resected left lung with two of the four *healed* lower lobe cavities. It shows two of the four lower lobe cavities, and the membranes as far as shown here are completely intact, smooth, shiny, glistening, and healed.

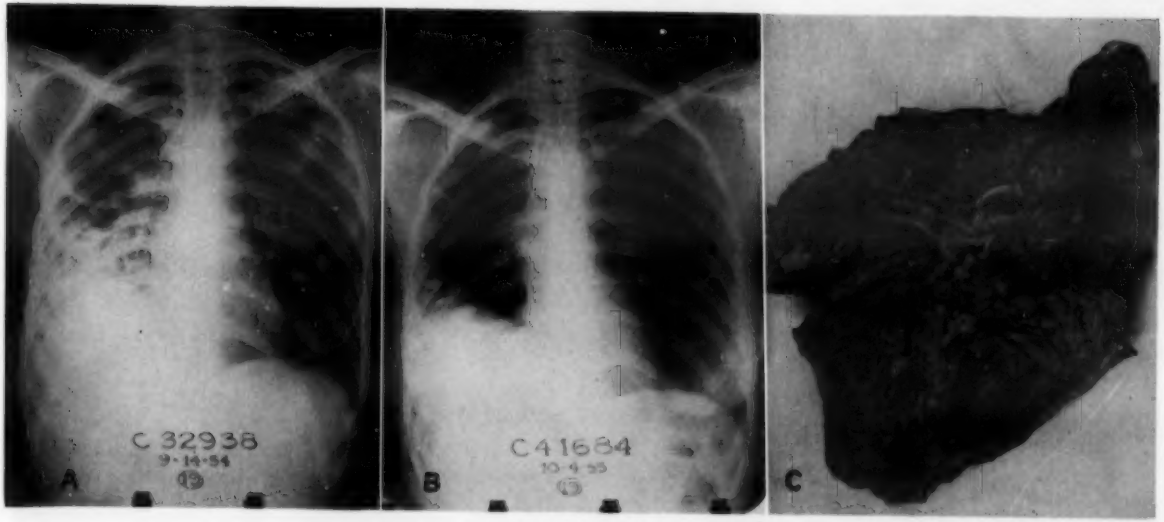


Case 1 (table 4)

FIG. 3*A*. Chest roentgenogram showing extensive bilateral disease on the patient's admission to the hospital, with right upper lobe cavitation.

B. Bronchogram outlining the right upper lobe cavity after long-term drug therapy.

C. Gross specimen showing right upper lobe cavity with an openly draining bronchus communicating directly with the open cavity. This was classified as a healing cavity because, even though the membrane was intact, smooth, shiny, and glistening throughout, there were a few small areas of caseation lying directly underneath the intact membrane. Some of the flecks up at the very top of this cavity can be seen.

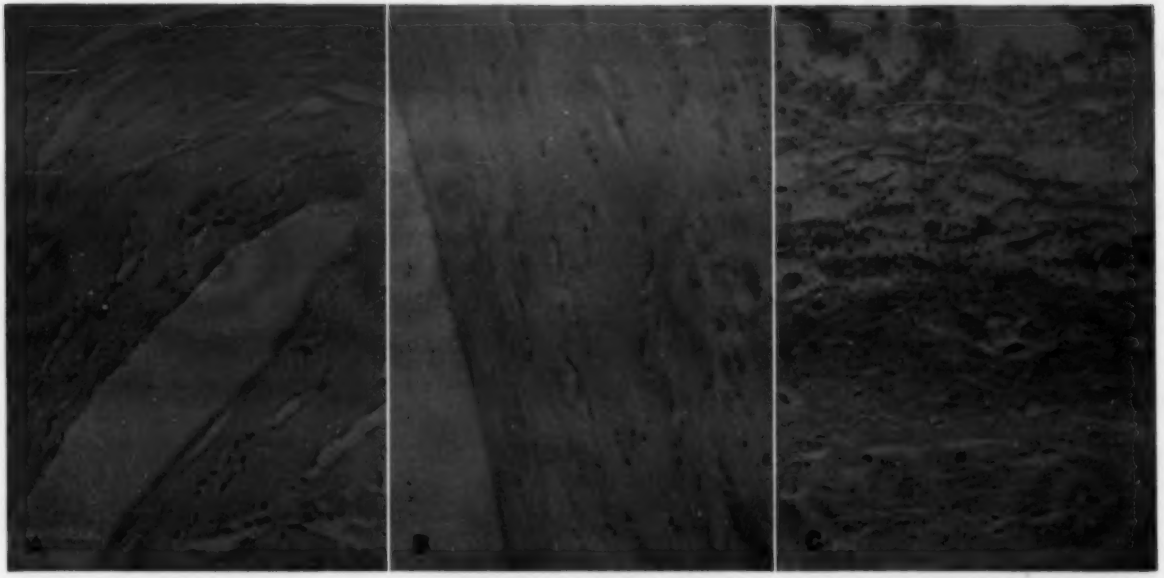


Case 13 (table 4)

FIG. 4*A*. Chest roentgenogram showing extensive bilateral disease with multiple cavities.

B. Chest roentgenogram showing marked clearing of the disease with drug therapy, the remaining destroyed lung containing multiple cavities.

C. Gross specimen showing three giant cavities, one in each lobe; the cavities in the upper and lower lobes were still healing and the middle lobe was healed.



Microscopic Pathology of Healed Cavities

FIG. 5 *A* and *B* reveal walls of typical healed cavities composed of hyalinizing connective tissue, lamellarly arranged with capillaries, totally lacking cellular infiltration.

C. This illustrates a hyalinizing cavitary wall with adjacent alveolar tissue showing the difference between a possible emphysematous bleb and a connective tissue wall of a healed cavity.

Microscopic Pathology of Healing Cavities

FIG. 6*A* exhibits a partially healing connective tissue wall in which a small tubercle and some distal calcium deposits with lymphocytic infiltrates are seen.

B shows an extensive accumulation of calcium deposits in the inner lining of the cavity wall, with scant lymphocytic infiltration.

C reveals the only re-epithelization encountered, directly at the bronchocavitary margin, seen in one of the healing cavities.

THE SYNDROME OF PERSISTENT CAVITATION AND NONINFECTIOUS SPUTUM DURING CHEMOTHERAPY AND ITS RELATION TO THE OPEN HEALING OF CAVITIES[1,2]

OSCAR AUERBACH AND MAURICE J. SMALL

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INTRODUCTION

There is probably no greater problem resulting from chemotherapy in tuberculosis than the pulmonary cavity which persists despite the absence of tubercle bacilli from the sputum. These cases are appearing with greater frequency because of two factors: (*1*) the prolonged use of antimicrobial agents and (*2*) the greater use of isoniazid. This problem was only rarely encountered before the use of specific drugs in the treatment of pulmonary tuberculosis.

The question arises of when the persistent cavity in the patient who is no longer discharging tubercle bacilli represents anatomic open healing. If such a case is one with a cavity lined by a necrotic zone, it is generally believed to be potentially dangerous. This view is based upon the demonstration that, if chemotherapy is interrupted in such cases, tubercle bacilli reappear in the sputum and these organisms are then generally resistant to the drugs given.

Another important question is the relationship between the clinical syndrome of persistent cavity without tubercle bacilli in the sputum and the pathologic open healing of the tuberculous cavity. Among the questions to be answered are: Is the presence of the clinical syndrome a forerunner of open healing; are there clinical, bacteriologic, and roentgenographic criteria to indicate that the lesions in these cases are anatomically healed; and do certain factors in chemotherapy, such as the duration of treatment and the selection of drugs (such as isoniazid), determine the frequency of occurrence of these cases of persistent cavitation with noninfectious sputum and the frequency of open healing?

Finally, what are the criteria necessary to establish the presence of either the clinical syndrome or open healing as determined by histopathologic examination?

METHODS AND MATERIALS

Criteria for the presence of the syndrome: The presence of an annular shadow in the roentgenogram and tubercle bacilli in the sputum is unequivocal evidence of a tuberculous cavity. The persistence of an annular shadow with the disappearance of tubercle bacilli from the sputum and gastric or tracheal washings are the criteria for the syndrome under consideration.

In some instances an annular density may appear at the site of a previously closed cavity after prolonged chemotherapy. Such a sequence of events may be the result of an inspissated cavity which has subsequently evacuated its contents, or it may be a cyst resulting from dilated air sacs which occur around the contracted closed cavity.

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[1] From the Laboratory and Tuberculosis Services, Veterans Administration Hospital, East Orange, New Jersey.

[2] Presented in part before the Fifteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, at St. Louis, Missouri, February 6-9, 1956.

TABLE 1

ANALYSIS OF RESECTIONS, JANUARY, 1953–DECEMBER, 1955

Total resections	235
Closed lesions	137
Cavitary lesions	98
Open-negative cases (bacteriologically negative cultures > 3 months preoperatively)	31
Pathologically open healed	11
Clinically open healed	20
Cavitary cases (bacteriologically positive at resection or negative less than 3 months)	67
Pathologically open healed	1
Pathologically "active"	66

Criteria of pathologic "open" healing: To demonstrate "open" healing of a tuberculous cavity so that there would be unanimity of opinion, one would have to fulfill the following criteria: that the cavity wall be transformed into a layer of connective tissue from which all signs of specificity had disappeared; that there be no tuberculous contents in the cavity; that no viable tubercle bacilli be recovered from the cavity wall or its contents. To satisfy the purists, it would also be necessary to examine the cavity by means of serial sections and at the same time macerate the cavity wall for bacteriologic studies.

For practical purposes it is the opinion of the writers that those cases should be accepted as "open" healing in which the lumen of the cavity is free of necrotic contents, the inner aspect contains no pyogenic membrane or necrotic lining, and the cavity wall contains no necrotic foci.

Analysis of material: In an attempt to study the question of "open" healing, the writers analyzed the pulmonary resections which were performed at the Veterans Administration Hospital, East Orange, New Jersey, from January 1, 1953, to December 31, 1955. During this period there were 235 resections for tuberculosis. Pathologic examination showed that closed lesions only were removed in 137 instances and a patent cavity in 98 cases (table 1).

Previous experience based upon examination of pathologic material had indicated that "open" healing could occur with as little as two or three months of chemotherapy. Accordingly, the cavities resected from those patients who had been in a noninfectious status for three months or longer were placed into the category of the "open-negative" syndrome. This was done without knowledge of the pathologic findings. Thirty-one cases fell into this category (table 1). Eleven of the 31 cases proved to be instances of pathologic open healing. One case of pathologic open healing occurred in which smears and cultures for tubercle bacilli had been negative for only two months; thus, there was a total of 12 cases of pathologic open healing in the 98 cavitary cases resected.

The cases were then divided into two groups: (*1*) the "open-negative" case, in which the cavity proved to be healed anatomically, which was designated as *pathologic open healing*, and (*2*) the cases in which tubercle bacilli could not be recovered by the usual methods for three or more months, but at the time of resection necrotic material lined the cavity; these were designated as *clinical open healing* (table 1). An analysis was then made of these two groups in an effort to uncover possible differentiating characteristics.

OBSERVATIONS

Duration of noninfectious status: The period during which sputum cultures were negative for tubercle bacilli in the pathologic open-healed group varied from two to thirty months, while in the clinical open-healed group it varied from three to sixty months. In this small series of cases there appeared to be no sig-

TABLE 2

DURATION OF BACTERIOLOGICALLY NEGATIVE CULTURES BEFORE RESECTION

Months	Number of Cases
In pathologic open healed (2 to 30 months)	
2–3	3
4–6	2
7–12	3
13–30	4
	12
In clinical open healed (3 to 60 months)	
3	5
4–6	9
7–12	5
13–60	1
	20

TABLE 3

CHEMOTHERAPY REGIMENS

In pathologically open-healed cases	12
Streptomycin-PAS	4
Isoniazid-containing regimens	8
In clinical open-healed cases	20
Streptomycin-PAS	6
Isoniazid-containing regimens	14

nificant difference between the two groups in this respect (table 2). Of extreme interest is the fact that in 6 of the 20 cases in noninfectious status for seven to sixty months there still remained a necrotic lining of the cavity. Thus, even though the present series is small, the writers believe that they are justified in stating that the duration of the period of noninfectious status is not a reliable indication that a cavity is anatomically healed.

Relationship to isoniazid therapy: As some investigators have expressed the opinion that open healing seems to be occurring more frequently since the use of isoniazid therapy, the present writers analyzed the material from this standpoint (table 3). Although 8 of the 12 patients with pathologic open healing did receive an isoniazid-containing regimen prior to resection, 14 of the 20 who had been noninfectious equally long proved to be not healed pathologically but had also received isoniazid-containing regimens. In the small group of cases isoniazid therapy did not seem to be a significant factor in increasing the incidence of pathologic open healing. It is believed that larger numbers of cases will have to be evaluated before this point is considered settled.

TABLE 4

CHEMOTHERAPY REGIMENS IN PATHOLOGICALLY OPEN-HEALED CASES

Regimen	Cases	Bacteriologically Negative Preoperatively
		months
Streptomycin-PAS	4	10–30
Isoniazid-streptomycin	4	3–14
Streptomycin-PAS (22) + Isoniazid (3)	1	>15
Isoniazid-PAS	1	5
Streptomycin-PAS (28) → Isoniazid-oxytetracycline (2)	1	2
Streptomycin-PAS (½) → Pyrazinamide-isoniazid (14)	1	13

→ Indicates change of regimen.
+ Indicates addition to regimen.
Numbers in parentheses indicate months of therapy.

TABLE 5

ROENTGENOGRAPHIC PATHOLOGIC CORRELATION

Thin-Walled Cystic Cavity and Pathologic Open Healing

		Months of Preoperative Bacteriologic Negativity	
		With Cystic Cavity	Without Cystic Cavity
Pathologic open-healed	4 of 12	7–14	2–30
Clinical open-healed	3 of 20	2–7	3–60
Open-positive	2 of 66	0	0

Preoperative chemotherapy: The preoperative chemotherapy regimens together with preoperative duration of bacteriologic negativity were analyzed in greater detail in the pathologic open-healed group (table 4). As noted, 8 of 12 patients in this group received isoniazid preoperatively, but in this connection several points bear mentioning. One patient was included in the isoniazid-treated group (line 3, table 4) who had received streptomycin-PAS for twenty-two months, followed by streptomycin, PAS, and isoniazid for three months prior to resection, with cultures negative for tubercle bacilli for at least fifteen months prior to resection. It is most doubtful that isoniazid was a factor in producing open healing in this case. Also of interest was one patient who received isoniazid and oxytetracycline for only two months (line 5, table 4), with prompt reversal of infectiousness as soon as this regimen was started, and showed pathologic open healing. This result would not have been predicted from the roentgenographic appearance (*see* figure 2) and the brief period of noninfectious status prior to resection. This patient had received streptomycin-PAS for many months without reversal of infectiousness prior to receiving isoniazid-oxytetracycline.

Relationship to roentgenographic findings: In view of the impression of several investigators that a thin-walled, cystlike annular lesion in the chest roentgeno-

gram of a tuberculous patient who has received prolonged chemotherapy and whose sputum cultures have failed to grow acid-fast organisms frequently indicates pathologic healing, the present writers have evaluated their data from this standpoint (table 5). In the pathologic open-healed group, 4 of the 12 cases presented such a cavity, and these had been in a noninfectious status for periods of seven to fourteen months. In the "clinical open-healed" group, only 3 of the 20 patients had such a cavity roentgenographically and these patients had cultures negative for tubercle bacilli for periods from two to seven months prior to resection. It is of interest that in 2 cases in the "open-positive" group thin-walled cystic cavities were present which were indistinguishable roentgenographically from those which proved to be anatomically healed. It would appear from this limited number of cases that, if an attempt is made to predict by roentgenographic and bacteriologic findings and by using the double criteria of cystic cavity and negative bacteriologic results for seven or more months, 4 of 5 cases fulfilling these criteria may actually prove to be instances of pathologic open healing. However, it should be stressed that two-thirds of the cases in the patho-

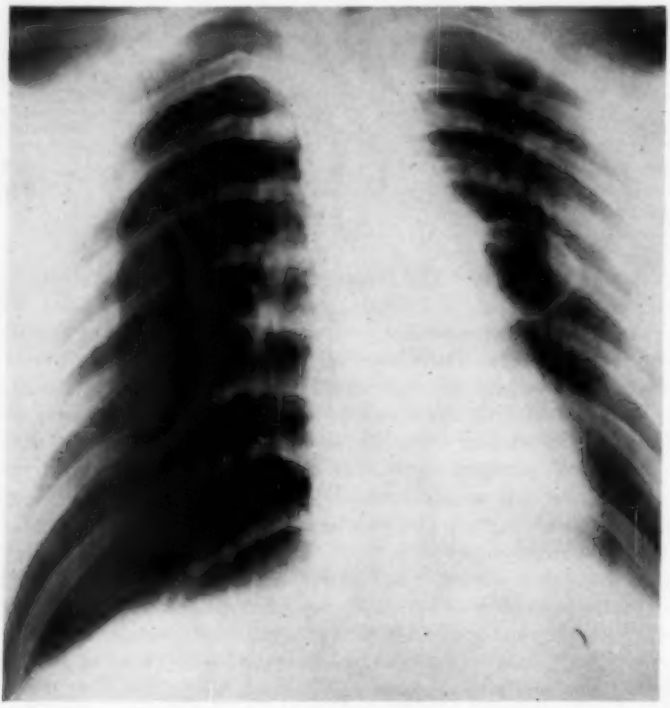

FIG. 1. Chest roentgenogram of patient with cultures negative for *M. tuberculosis* for nine months prior to resection of thin-walled cystic-appearing cavity in left lung. Possibility of pathologic "open" healing of cavity predicted but not found.

FIG. 2. Chest roentgenogram of patient with cultures negative for *M. tuberculosis* for only two months at the time of resection of irregular thick-walled excavations in the left upper lobe. Pathologic "open" healing of cavity definitely not expected but found.

logic open-healed group did not present such a cavity by roentgenographic study.

The pitfalls of attempting such a prediction are illustrated by the following 2 cases. One patient (figure 1) had had cultures negative for tubercle bacilli for nine months preoperatively and had had a thin-walled cystlike cavity for many months prior to resection. It was the impression of the writers that this cavity would probably show pathologic open healing, but such was not proved to be the case. A second patient (figure 2) with known chronic pulmonary tuberculosis for at least four years, including many months of unsuccessful treatment with streptomycin-PAS, had been under treatment with isoniazid and oxytetracyline for only two months, with prompt reversal of infectiousness as soon as this regimen was started. This fact, plus the roentgenographic appearance, certainly led to a prediction of no open healing. Such, however, did not prove to be the case (figure 3).

Rapid closure of a giant cavity which revealed pathologic open healing, with

FIG. 3. *Open healing following chemotherapy:* Photomicrograph of cavity showing a layer of hyalinized connective tissue (*A*) in which there is a complete loss of cellular elements. A zone of loose connective tissue (*B*) forms the outer wall of the cavity.

spontaneous reopening after one year of pyrazinamide-isoniazid therapy (apparently due to distension by a bronchial mechanism) is of great interest and was observed in one case in the pathologic open-healed group.

The patient, a 21-year-old Negro male, was placed on pyrazinamide-isoniazid treatment when pulmonary tuberculosis, with a giant cavity in the right middle lobe, was discovered (figure 4). Reversal of infectiousness occurred promptly and the large cavity appeared to have healed by a linear scar (figure 5). This was confirmed by anteroposterior and lateral tomograms obtained during the fourth month of therapy. Serial monthly chest roentgenograms showed sudden reopening of the large cavity (figure 6) at the twelfth month of therapy with the appearance of a fluid level. This was associated with malaise and low-grade fever but with repeated negative bacteriologic examinations. The differential diagnosis at that time included reopening of a healed tuberculous cavity, a loculated empyema, and a nontuberculous infected cyst. During resection of the cavity, which replaced almost the entire middle lobe, the surgeon noted that the communicating bronchus entered the cavity at a markedly acute angle and that in the resting (expiratory) position the communicating bronchus appeared to be markedly constricted.

Pathologic examination: Pathologic examination of the resected middle lobe showed almost all of the middle lobe occupied by two cavities. One measured 4 cm. by 3 cm., and the other 4.5 cm. by 4.5 cm. The walls of the cavities measured one millimeter in thickness and their linings were smooth and gray (figure 7*A*).

Microscopically the cavities in all aspects were lined by hyalinized connective tissue. In one area this gave way to the lumen of a bronchus. At the bronchocavitary junction the epithelium of the bronchus was squamous in character and extended along the inner surface of the cavity for a short distance (figure 7*B*).

Patient H.B.

FIG. 4 (Left). Chest roentgenogram obtained on October 1, 1954, at the time when pyrazinamide-isoniazid therapy was started. Note large cavity in region of right middle lobe adjacent to cardiac border.

FIG. 5 (Center). Chest roentgenogram obtained on May 23, 1955, after seven months of pyrazinamide-isoniazid therapy. Smears and cultures obtained after the second week of therapy were consistently negative for *M. tuberculosis*. The right middle lobe cavity was quickly lost to view and was considered as having healed by linear scar seen on this film as horizontal linear density overlying the fourth right anterior rib.

FIG. 6 (Right). Chest roentgenogram obtained on November 7, 1955, showing sudden reopening of right middle lobe cavity with appearance of fluid level. Pyrazinamide had been discontinued on July 5, 1955, but isoniazid therapy was maintained. Numerous smears and cultures continued negative for *M. tuberculosis* in the presence of this large cavity which proved to be pathologically "open" healed.

FIG. 7A. *Open healing following chemotherapy:* Coronal section of the right middle lobe showing two cavities (*A* and *B*) with a smooth lining. The upper cavity (*A*) is partially collapsed in this view.

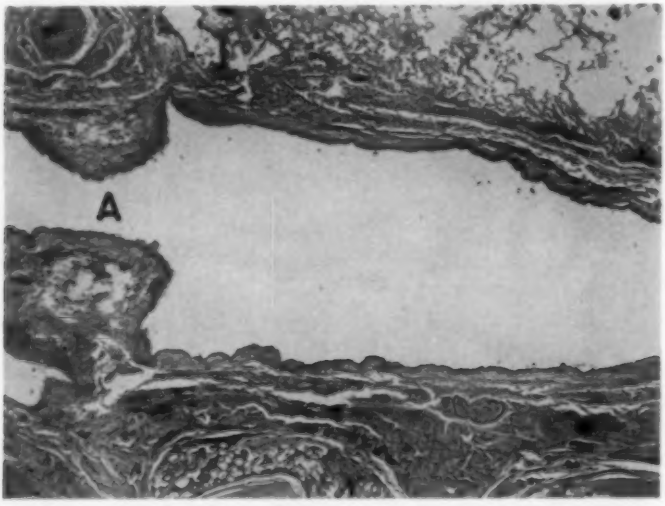

FIG. 7B. Photomicrograph of the cavity (figure 7*A*) shows a thin wall composed of hyalinized connective tissue. A patent bronchus with a narrowed lumen (*A*) communicates with the cavity. Intact lung parenchyma surrounds the cavity.

Significance of acid-fast bacilli on microscopy with negative cultures: The significance of the finding of acid-fast bacilli on microscopy but not on culture is of extreme interest. In this series of patients with cavities, 3 continued to discharge acid-fast bacilli in the sputum, as determined by microscopy, long after the

sputum had become negative for tubercle bacilli on culture. In one case the cultures had been "negative" for five months and smears for only one month at the time of resection; and, in another, cultures had been "negative" for nine months and smears for only two months at the time of resection. The latter case is of particular interest since the cavity presented in the roentgenogram was a typical thin-walled cystic one. It is believed that this is analogous to the finding of stainable acid-fast bacilli in resected specimens which do not grow by routine culture methods, and that it is erroneous to neglect the importance of smears of concentrated sputum in chemotherapy studies.

Discussion

The findings in the present study suggest that the presence of the syndrome of persistent cavitation with noninfectious sputum does not necessarily indicate that the cavity present is anatomically healed. An evaluation of the material indicates that the acceptance of the syndrome as representing the presence of a healed cavity is extremely hazardous. This is emphasized by the finding of sputum cultures negative for tubercle bacilli from two to six months prior to resection in 5 of the 12 patients who showed anatomic healing. But in 6 of 20 cases, the resected cavity showed the presence of a necrotic lining in spite of the absence of tubercle bacilli from the sputum for seven to sixty months.

Several reports in the literature have demonstrated cases of pathologic open healing and seem to imply that instances of the syndrome of "open-negative" cavities which were not resected were also instances of pathologic open healing. Thus, the impression appears to have been gained that open healing of tuberculous cavities is much more frequent than the present writers have been able to demonstrate in the gross and microscopic studies of the cavities in the resected material.

The problem of the open-negative case and its relationship to pathologic open healing is one which occurs primarily as a result of prolonged chemotherapy. A study of the available literature referable to the processes of open healing without the benefit of antimicrobial agents shows a small number of such reports (1–21). The great rarity of this occurrence in the era before chemotherapy is emphasized by the fact that Pagel and Simmonds (21) found that only five such cases had been reported up to 1942. Since then the present writers have been able to find only an occasional report of this kind before chemotherapy became an important adjunct in the treatment of chronic pulmonary tuberculosis (22, 23). The rarity of open healing is emphasized by the fact that in more than 2,000 autopsies of patients with chronic pulmonary tuberculosis, when no antimicrobial drugs were given during life, only two cases of pathologic open-healed cavities were observed (20). It was the impression then of the present writers that an important factor in the process of open healing was a wide bronchocavitary junction.

The present writers have been impressed (24), as have others (25–34), by the observation that open healing seems to be observed with increasing frequency after long-term chemotherapy. Cavity healing with inspissation, however, is still a much more frequent occurrence.

In order to answer some of the questions concerning the processes of open healing, it is believed that a review of the pathology of the tuberculous cavity and the differences brought about by chemotherapy as well as the changes which lead to open healing may be pertinent.

A fully developed tuberculous cavity which has just formed and which has not had the benefit of chemotherapy has a characteristic appearance. The inner lining is composed of a necrotic zone of variable width. As this zone of necrosis is evacuated, a narrow layer of fibrin containing neutrophils forms the inner wall. This layer has been designated as the pyogenic membrane and is the result of exuding fibrin and neutrophils from the zone of vascular granulation tissue. Beyond the pyogenic membrane and area of necrosis is a layer of vascular granulation tissue which is composed of many dilated capillaries, epithelioid cells, fibroblasts, giant cells, and occasional collagen fibrils. This layer in turn is surrounded by a narrow zone of loose connective tissue representing the organization of the inner layer of perifocal reaction. The zone of connective tissue in the outer portion of the wall becomes wider as the perifocal reaction is converted into connective tissue.

The gross pathology of a cavity showing open healing demonstrates that the inner surface of the cavity is lined by a red or gray surface which is smooth. Necrotic particles are absent from the inner wall of the cavity. The cavity walls are thick and fuse with the overlying pleura which is also greatly thickened (figure 8*A*). Bronchi open widely into these cavities and are lined by a smooth surface. Trabeculae representing obliterated pulmonary arteries also have a smooth, red or gray surface.

On microscopic examination, the process of healing is characterized by invasion of fibroblasts and epithelioid cells from the zone of granulation tissue into the inner necrotic zone and the pyogenic membrane. The zone of vascular granulation tissue shows an increase of connective tissue fibers at the expense of the capillaries and the cellular elements. As the inner zone is invaded and replaced by the elements of repair, epithelium from the bronchial wall may extend onto the cavity wall. In the experience of the present writers, epithelial regeneration never extends over the necrotic lining or the pyogenic membrane. The necrotic contents of the cavity lumen are completely evacuated through the widened bronchial outlet.

As healing progresses, the inner and middle layers of the cavity wall are occupied by granulation tissue, which shows an ever increasing number of collagen fibrils with a concomitant decrease in both cells and capillaries until finally a concentric zone of hyalinized connective tissue remains from which either all or nearly all specific tuberculous elements have disappeared (figure 8*B*). This zone of hyalinized connective tissue is surrounded by a still wider area of loose connective tissue representing the organized perifocal reaction. The latter zone contains many compressed and distorted alveoli lined by cuboidal cells. This area in some regions fuses with the overlying thickened pleura, and in other regions is surrounded by dilated air sacs.

The bronchial ulcer at the bronchocavitary junction is invaded and replaced

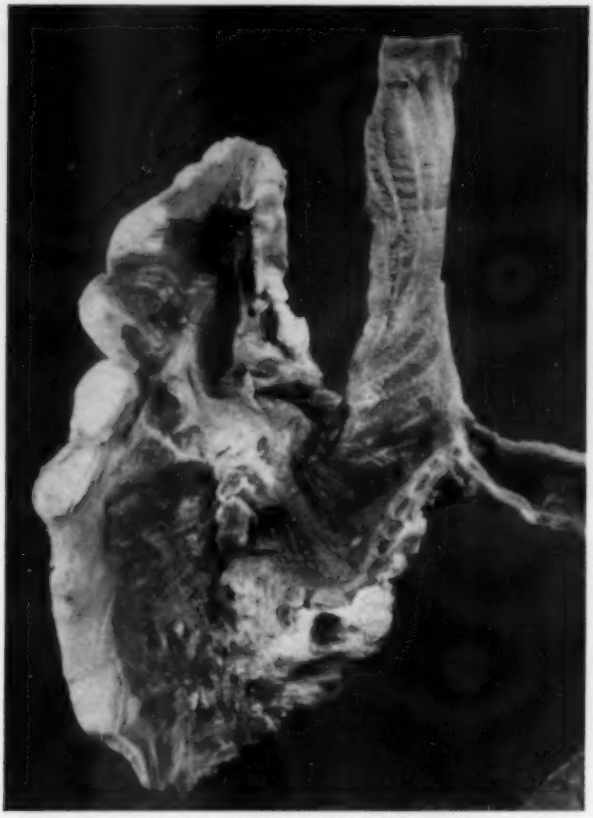

FIG. 8A. *Open healing without chemotherapy:* Coronal section of the right lung shows a thick-walled cavity occupying most of the right upper lobe. The inner lining of the cavity is smooth. Bronchi open widely into the cavity.

by granulation tissue. As this occurs, regeneration occurs from the intact epithelial lining proximal to the ulcer. The regenerated epithelium is generally flattened or squamous in character.

The question often is asked, "what determines the extent to which epithelium extends on to the cavity wall?" It is the opinion of the writers that there are several factors which determine its extent. As has already been stated, epithelium will not regenerate over a surface which is lined by a pyogenic membrane or necrotic lining nor will it extend onto a surface lined by connective tissue. Thus, if the cavity wall has been transformed into connective tissue before the epithelial regeneration has extended onto it, it can no longer be epithelized. For the epithelial extension to cover a part or all of a cavity wall, the process of regeneration must extend onto the area of granulation tissue adjoining it. If in

FIG. 8*B*. Photomicrograph of the cavity wall (figure 8*A*) shows it to be composed of dense connective tissue from which all signs of tuberculosis have disappeared.

the course of regeneration the inner wall of the cavity is lined by a necrotic zone or a connective tissue layer, epithelium will stop abruptly at this point. Since the process of healing is an uneven one, open-cavity healing with complete epithelization is a very infrequent occurrence, indeed.

It has previously been pointed out (35) that an important factor in the increase in the number of cases of open healing after long-term chemotherapy is the re-epithelization of the bronchus at the bronchocavitary junction. In untreated cases the check-valve mechanism results in a retention of the necrotic contents within the cavity. As a result of the healing of the ulcerated bronchial surface and re-epithelization, wider drainage is now possible. Another important factor is that under prolonged chemotherapy the processes of progression halt from within and from without.

Thus chemotherapy performs three functions to aid in open healing of tuberculous cavities: (*1*) It halts the process of cavity progression from within and without. (*2*) It favors the re-epithelization of the bronchus at the bronchocavitary junction and thus widens the outlet for the evacuation of the necrotic contents from the cavity lumen. (*3*) It accelerates healing of the cavity wall, so that it is more rapidly converted into hyalinized connective tissue.

Open healing under chemotherapy may be primary or secondary. In the primary process re-epithelization at the bronchocavitary junction results in a widening of the bronchial outlet. In the secondary form, re-epithelization of the bronchocavitary junction interrupts the check-valve mechanism and results in a closure of the cavity lumen with inspissation of the cavity contents. Subsequently there may be a liquefaction of the necrotic contents within the cavity

lumen. Neutrophils intermingled with mucin may extend from the lumen of the bronchus into the inspissated cavity among the necrotic fragments with a resultant sequestration, liquefaction, and evacuation through the bronchial lumen.

Comparing the open-healed cavity wall after chemotherapy with that healed without the benefit of drugs, it is found that in the former instance the cavity is much thinner as a result of a narrow or absent zone of loose connective tissue in the outer wall (*see* figure 7*B*), while the latter has a wide layer of fibrous tissue, frequently laden with coal pigment (figure 8*B*). After chemotherapy such healed cavities may be mistaken for lung cysts or emphysematous blebs. It is believed that such areas may be accepted as open-healed cavities if they occupy the site of a cavity visualized in the roentgenograms, if bronchi communicate with them, and if they do not surround an inspissated cavity or a radial scar.

Summary

Open healing of tuberculous cavities, rarely observed in the era before chemotherapy, is observed with greater frequency in patients who receive prolonged chemotherapy. Only 2 cases of pathologic open healing were found in more than 2,000 autopsies prior to the advent of antituberculous chemotherapy; 12 such cases occurred in 98 cavities resected following chemotherapy.

Thirty-one of the 98 cases of cavity were classified as "open negative" and met the criteria of the persistence of annular shadow and the disappearance of tubercle bacilli from the sputum by conventional culture methods for a period of three or more months prior to resection.

Eleven of the 31 cases showed pathologic "open" healing: the necrotic contents of the cavity and the necrotic lining of the cavity wall had been evacuated. The cavity wall had been transformed into connective tissue with a disappearance of all signs of specificity (tuberculous granulation tissue). A twelfth case showed anatomic healing only two months after tubercle bacilli had disappeared from the sputum.

Instances of the clinical syndrome "open-negative case" in which anatomic healing was not subsequently demonstrated were compared with 12 cases of anatomic "open" healing. No significant differences could be ascertained between the two groups with respect to duration of noninfectious status before resection, duration or type of chemotherapy, and roentgenographic findings.

Prolonged chemotherapy (*a*) aids in the re-epithelization at the bronchocavitary junction and results in a permanent communication between the cavity and the exterior. Thus, even after inspissation of the cavity contents, late liquefaction may result in evacuation through the communicating bronchus; (*b*) the re-epithelized bronchus results in a widened outlet, so that the cavity contents may be more easily evacuated; (*c*) by its action on the tubercle bacilli within the cavity lumen, prolonged chemotherapy prevents further necrosis of the cavity wall and thus enhances the healing changes.

Cavity walls are much thinner after prolonged chemotherapy than after little or no chemotherapy. Thin-walled cysts resulting from contracting tuberculous foci are difficult to distinguish anatomically from open-healed tuberculous cavi-

ties, especially in those lesions which have been under the influence of prolonged chemotherapy from soon after their development.

Sumario

La Caverna Tuberculosa Negativa Abierta en el Transcurso de la Quimioterapia y su Relación con la Cicatrización Abierta

La cicatrización abierta de las cavernas tuberculosas, rara vez observada en la era anterior a la quimioterapia, se observa con mayor frecuencia en los enfermos que reciben quimioterapia prolongada. En más de 2,000 autopsias anteriores al advenimiento de la quimioterapia antituberculosa, sólo se descubrieron 2 casos de cicatrización abierta patológica, hubo 12 de esos casos en 98 cavernas resecadas consecutivamente a la quimioterapia.

Treinta y uno de los 98 casos de cavernas fueron clasificados como negativos abiertos y cumplieron las pautas de persistencia de la sombra anular y desaparición de los bacilos tuberculosos del esputo conforme a las técnicas de cultivo aceptadas durante un período de tres meses o más antes de la resección.

Once de los 31 casos revelaron cicatrización abierta patológica: el contenido y el revestimiento esfacelados de la caverna habían sido evacuados. La pared de la caverna se había transformado en tejido conjuntivo, con desaparición de todos los signos de especificidad (tejido de granulación tuberculoso). Un duodécimo caso mostró cicatrización anatómica únicamente a los dos meses de desaparecer los bacilos tuberculosos del esputo.

Veinte cavernas negativas abiertas, que no habían cicatrizado anatómicamente, fueron consideradas como casos de cicatrización abierta clínica, comparándose con los 12 casos de cicatrización abierta patológica; no pudieron descubrirse diferencias entre los dos grupos en lo tocante a duración de los cultivos negativos antes de la resección, duración o forma de la quimioterapia y hallazgos radiográficos.

La quimioterapia prolongada (*a*) ayuda en la reepitelización en la unión broncocavitaria y da por resultado una comunicación permanente entre la caverna y el exterior, de modo que, aun después de espesarse el contenido de la caverna, la licuación tardía puede acarrear vaciamiento a través del bronquio de comunicación; (*b*) el bronquio reepitelizado ofrece un desagüe ensanchado, de manera que es más fácil evacuar el contenido de la caverna; (*c*) por su efecto sobre los bacilos tuberculosos del interior de la caverna, la quimioterapia prolongada impide el esfacelamiento ulterior de la pared de la caverna, acrecentando así las alteraciones cicatrizantes.

Las paredes de las cavernas son mucho más delgadas después de la quimioterapia prolongada que cuando ésta es poca o nula. Es difícil distinguir anatómicamente los quistes de paredes delgadas derivados de la contracción de los focos tuberculosos de las cavernas tuberculosas cicatrizadas abiertas, sobre todo en las lesiones que han recibido quimioterapia prolongada poco después de su aparición.

Resume

La caverne tuberculeuse ouverte avec bacilloscopie négative au cours de la chimiothérapie et sa corrélation avec l'epithélialisation

L'épithélialisation des cavernes tuberculeuses ouvertes, rarement observée dans l'ère pré-chimiothérapeutique, est constatée avec une plus grande fréquence chez les malades soumis à la chimiothérapie prolongée. Avant l'introduction de la chimiothérapie antituberculeuse, il n'a été découvert que 2 cas de cicatrisation "pathologique" de cavernes (c'est à dire, bacilloscopie négative, mais présence de matière caséeuse) sur 2.000 autopsies: 12 de ces cas apparaissaient dans 98 cavernes réséquées après chimiothérapie.

Sur les 98 cavernes réséquées, 31 cas furent classifiés dans la catégorie des cavernes épithélialisées avec bacilloscopie négative, c'est-à-dire qu'ils satisfaisaient aux critères de la persistance d'une opacité annulaire et disparition des bacilles tuberculeux dans l'expec-

toration dans les conditions culturales classiques pendant une période de trois mois ou plus avant la résection.

Sur ces 31 cas, 11 présentaient les caractères de la cicatrisation ouverte pathologique: le contenu caséeux de la cavité et les tissus nécrosés de la paroi de la caverne étaient évacués. La paroi des lésions cavitaires était transformée en tissu conjonctif avec disparition de tout signe de spécificité (tissu de granulation tuberculeuse). Dans un douzième cas, la cicatrisation anatomique n'est apparue que deux mois après la disparition des bacilles tuberculeux dans les crachats.

La classification cicatrisation clinique de la caverne ouverte est appliquée dans 20 cas de cavernes ouvertes avec bacilloscopie négative, et ils sont confrontés avec 12 cas de cavernes ouvertes pathologiques (pathologic open healing). Il n'a pu être établi aucune différence entre les deux groupes en ce qui concerne la durée des cultures négatives avant résection, la durée ou le type de la chimiothérapie et les signes radiologiques.

La chimiothérapie prolongée: a) facilite la ré-épithélialisation au niveau de la jonction broncho-cavitaire et assure une communication permanente entre la cavité et l'extérieur, de sorte qu'après desiccation du contenu cavitaire, la liquéfaction ultérieure permet l'évacuation par la bronche communicante; b) la bronche ré-épithélialisée constitue une ouverture élargie facilitant l'évacuation du contenu cavitaire; c) par son action sur les bacilles tuberculeux à l'intérieur de l'excavation, la chimiothérapie prolongée prévient l'accroissement du processus nécrotique au niveau de la paroi et favorise ainsi la cicatrisation.

A la suite de la chimiothérapie prolongée, les parois cavitaires sont beaucoup plus minces que lorsque la chimiothérapie a été brève ou absente. Du point de vue anatomique, il est difficile de distinguer les kystes à paroi mince résultant d'un foyer tuberculeux contracté des cavernes tuberculeuses épithélialisées, en particulier dans les lésions ayant été précocement soumises à une chimiothérapie prolongée.

Acknowledgment

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BILATERAL RESECTION IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1,2]

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INTRODUCTION

Between January, 1949, and December, 1955, approximately 4,000 patients were admitted to the West Tennessee Tuberculosis Hospital in Memphis. These patients were all admitted to the medical service and treated according to the methods in vogue at the time of their admission. Surgical consultation was sought for all patients in whom it appeared advisable, and surgical therapy was applied when it was necessary in the judgment of the hospital staff. The operative procedures employed have varied through the years, thoracoplasty, pneumonolysis, phrenic paralysis, and plombage gradually giving way to resection.

Approximately 900 pulmonary resections were performed on the surgical service during the period under discussion. With the advent and subsequent refinements of chemotherapeutic management, there has been a gradual change in the surgery, the constant trend being toward the removal of less and less tissue from more and more patients. Early tuberculosis in the lung usually responds dramatically to bed rest and drug therapy, and the removal of residual foci forms the majority of surgical procedures at the present time.

In the management of residual disease foci, the bilateral case is of particular interest. Even though pulmonary resection has become well standardized and remarkably safe, an appreciable morbidity, a slight mortality, and a measurable encroachment upon the respiratory reserve remain, so that only a few patients are eligible for excision of diseased areas from both lungs. During the time in which 900 patients had pulmonary resection, bilateral surgery was recommended in only 60. The bilateral program was carried out in 33 patients, but in 27 others the plan was abandoned at some stage for one reason or another. A review of the 60 cases forms the basis for this report.

OBSERVATIONS

Characteristics of group: The age range of the patients may be seen in table 1. The majority of those in whom both sides were operated upon were less than forty years old. In fact, of 19 patients more than forty years of age who were recommended for bilateral surgery, only 8 had the program completed. Most people in this age group, and especially the old men who comprise such a large part of the current tuberculosis problem, have too much pulmonary damage to permit bilateral resection.

[1] From the West Tennessee Tuberculosis Hospital and University of Tennessee Medical School, Memphis, Tennessee.

[2] Presented before the Medical Session, as part of *Section 5B*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 23, 1956.

TABLE 1

TUBERCULOUS PATIENTS RECOMMENDED FOR BILATERAL PULMONARY RESECTION ACCORDING TO COMPLETION OF PROGRAM BY AGE GROUPS

Age Group	Completed	Not Completed
years		
0–19	3	4
20–39	22	12
40 and older	8	11
Total	33	27

TABLE 2

REASONS FOR NOT COMPLETING PROGRAM OF PLANNED BILATERAL PULMONARY RESECTION

Reason	Number of Patients
Refused all surgery	3
Refused surgery on second side	1
Surgery on second side unnecessary	13
Low respiratory reserve	6
Operative complication, first side	3
Died	1
Total	27

TABLE 3

DURATION OF TUBERCULOSIS IN PATIENTS RECOMMENDED FOR BILATERAL PULMONARY RESECTION

Duration	Surgery Completed	Not Completed
months		
1–12	13	2
12–36	2	8
36–48	4	0
48 and older	14	14
Total	33	24

The reasons for failure to complete the bilateral program are outlined in table 2. Operation on the second side was considered unnecessary in 13, either because significant residual was left from the first operation and the two sides appeared equally diseased, or because the lesion in the second side improved more than could be predicted. In 9 patients, lowered respiratory reserve or persistent operative complications made the surgery on the second side inadvisable.

The duration of disease for the two groups of patients may be seen in table 3. Among the 24 patients, 9 did not have the second operation because of complications or pulmonary insufficiency following their first surgery. Eight of these

TABLE 4

EXTENT OF PULMONARY RESECTION IN TUBERCULOUS PATIENTS HAVING BILATERAL SURGERY

Extent of Resection	Number of Patients
Partial lobectomy, bilateral	11
Lobectomy and contralateral partial lobe	15
Lobectomy, bilateral	3
Lobectomy, bilateral plus unilateral partial	4
Total	33

9 had disease of more than four years' duration. Regression of disease made operation on the second side unnecessary in most of the lesions less than three years old, but in only 4 of 14 patients with lesions of more than four years' duration.

Extent of pulmonary excision: The extent of pulmonary excision is shown in table 4. Almost all of the resections were in pulmonary units, either lobe, segment, or subsegment, with wedge excision quite rare. Two-thirds of the patients lost at least one lobe, while 7 of 33 had bilateral lobectomy. Four had additional segmental resection on one side or the other. The 11 others had purely segmental resection in all sorts of combinations. One woman has had at least one segment removed from all five of her lobes. Contralateral resection after pneumonectomy has not been done.

Indications for bilateral resection: The indications for resection in these patients were the same as in all tuberculous patients, with the provision that the disease should be so localized as to make resection on both sides possible. Nodular residuals, open cavity, and bronchial destruction were the most frequent indications. Bronchograms were used routinely to map out the diseased segments and their anatomic variations. Laminagrams and other specialized roentgenographic techniques were employed from time to time. A careful evaluation of pulmonary function was done on each patient, with exercise tolerance serving probably as the most important test. Within the limits of resection as reported here, respiratory function has not been unduly restricted by the removal of pulmonary tissue, but has on occasion been severely compromised by pleural complications.

Operative schedule: The conduct of the operative procedure is not radically different from any other pulmonary resection, except that the condition of the opposite lung influences decisions as to the amount of tissue to be removed. It is advisable to rid the lung of all palpable disease. At times, however, it becomes necessary to remove only the most significantly involved segments from the first side in order to preserve enough pulmonary tissue to make operation on the second side possible in the future.

The second operation is performed ten to twenty-four weeks following the first, depending upon the rapidity of recovery, the state of pulmonary function, and the response to continued therapy of the lesions in the remaining lung. As

TABLE 5

SURGICAL COMPLICATIONS IN PLANNED BILATERAL PULMONARY RESECTIONS ACCORDING TO COMPLETION OF PROGRAM

Complications	Number of Complications	
	66 operations 33 patients	24 operations 24 patients
Bronchopleural fistula	7	6
Postoperative spread of tuberculosis	3	0
Atelectasis	1	0
Dyspnea	1	2
Total number of complications	12	8

indicated previously, in some instances, frequently the second operation becomes unnecessary.

Complications of surgery: The surgical complications in the group of 57 patients are listed in table 5. Among the bilateral surgery patients, there was no difference in the frequency of complications on the first side or on the second side, on the right side or on the left side. The incidence was greater in the 24 who had only one operation (33 per cent) than in those who had bilateral procedures (18 per cent).

Examination of resected specimens: The histopathology of the bilateral specimens has been reviewed. In general, the histologic reaction is the same in both sides. The tuberculous process shows a slight quantitative change, depending principally upon the interval between the two operations. The lesions on the second side show slightly more fibrosis and appear less active. Cavities when present bilaterally appear quite similar in activity and in components of the cavity wall. No example of a healed cavity was discovered. The secondary changes, such as emphysema and focal fibrosis related presumably to the healing of tuberculous lesions, were also remarkably similar on the two sides. The amount of compensatory emphysema was almost identical, as was the degree of fibrosis and contraction of pulmonary parenchyma adjacent to tuberculous lesions.

Peribronchial fibrosis and inflammation when marked on one side were found to be almost identical on the second side, with a little more fibrosis and a little less inflammation in the side that had longer treatment. In a few cases, bronchial distortion and dilatation were more marked in the second side. Even though two years had elapsed between resections in several of the patients, the reactions in the lung remained qualitatively similar. It appears therefore, that, to some extent, the pathologic findings (gross and microscopic) in the first side help determine whether the other side need be operated upon. This factor militates against consideration of simultaneous bilateral surgery.

A search for acid-fast organisms has been made in every surgical specimen. Despite the fact that sputum cultures have in most cases been negative for tubercle bacilli at the time of surgery, the bacilli were found in 43 of 66 speci-

TABLE 6

PRESENT STATUS OF PATIENTS FOR WHOM BILATERAL PULMONARY RESECTION WAS ONCE PLANNED

Status	Bilateral Surgery	Unilateral Surgery
	Number	*Number*
Satisfactory	24	16
Limited capacity	4	6
Unsatisfactory	4	1
Dead	1	1
Total	33	24

mens. In 13 instances, acid-fast bacilli were present only in the material removed from the first side. In 15 patients, the specimens from both sides were positive for acid-fast bacilli, and in only 5 patients were the tissue examinations negative on both sides. Despite considerable care and attention to details of culture media and methods, no consistency had been achieved in the writers' laboratory in culturing these organisms.

Present status: The present condition of the patients six months to five years postoperatively is tabulated in table 6. The term *unsatisfactory* includes those with gross residual disease, cavitation, tubercle bacilli in the sputum, or a persisting surgical complication. The satisfactory results are slightly higher in those in whom the surgical program was completed. The patients who were limited by pulmonary insufficiency were observed to be more numerous than the patients who had the operation on only one side. Four patients have had bilateral surgery and still have tubercle bacilli in the sputum. There has been one death from tuberculosis in the unilaterally operated group. The one death in the bilaterally operated group was from cirrhosis of the liver.

Modifying factors: The age of the patient and the duration of his disease have a marked influence upon the outcome following surgery. The occurrence of surgical complications is even more important. Six of the eight unsatisfactory results in the patients who completed their operative program occurred in the 10 who had operative complications. Only 2 patients with unsatisfactory results are among the 23 who had no complications accompanying surgery. The importance of complications is further emphasized by the 3 patients whose second operation was prevented by the occurrence of major complications of the first procedure.

DISCUSSION

In a consideration of bilateral pulmonary resection for tuberculosis, one is impressed by the relative infrequency of the procedure. During the time in which more than 900 patients were treated with unilateral surgery, only 33 had operations on both sides. In these 33 the results, on the whole, are satisfactory, especially in view of the fact that the disease was judged to be of sufficient severity to warrant the second operation. The second side in each instance

was considered as a new problem, and the decision to operate was influenced by the findings at the time of the first surgery. The gross and microscopic findings in the 66 operative specimens have been reviewed, and there are only 2 of the 66 which suggest that surgery had been unnecessary. One of these was a specimen from the first side operated upon and the other was from the second side. The general similarity of the histologic reaction to tuberculosis in the two lungs of the same patient was of considerable interest.

The 24 patients who had one operation, but not the other, have essentially the same control of their tuberculosis as those with both sides operated upon. Sufficient time has not yet elapsed to permit conclusions from this fact, since many of the patients are continuing chemotherapy.

In practice, patients with long-standing bilateral disease and reduced pulmonary function, who are more than forty, can only complete a bilateral surgical program in an exceptional instance. The lung is usually damaged more than roentgenography suggests, and anatomic planes are poorly defined. These factors increase the liability to surgical complications, and the first side is frequently all that can be operated upon.

In younger persons, whose disease responds well to chemotherapy and rest but who have persistent significant disease, resection from both lungs can be expected to be successful and to leave the patient with good pulmonary function and with lessened likelihood of subsequent relapse of tuberculosis.

Summary

Bilateral resection for pulmonary tuberculosis was advised in 60 patients at the West Tennessee Tuberculosis Hospital. The bilateral program was completed in 33 patients, while, in 27, the full program of planned surgery was not completed. A review of the outcome in these patients and a comparison of the two groups show that results obtained have been generally satisfactory. There is a qualitative similarity in the pathologic response in the two lungs of the same person, so that the microscopic findings in the first operation can be used to determine the necessity for operation on the second side.

Bilateral resection is tolerated well by patients in younger age groups, but those more than forty are prone to develop complications which severely limit respiratory function.

Sumario

La Resección Bilateral en el Tratamiento de la Tuberculosis Pulmonar

A 60 enfermos del Hospital para Tuberculosis del Oeste de Tennessee se les aconsejó la resección bilateral por tuberculosis pulmonar. En 33 se llevó a cabo por completo el plan bilateral, mientras que, en 27, no se terminó. Un repaso del desenlace en estos enfermos y una comparación de los dos grupos demuestran que los resultados obtenidos han sido generalmente satisfactorios. Existe una semejanza cualitativa en la respuesta histopatológica en los dos pulmones de la misma persona, de manera que cabe usar los hallazgos microscópicos de la primera operación para determinar la necesidad de la intervención en el otro lado.

La resección bilateral es bien tolerada por los enfermos de los grupos de edad más joven, pero los de más de cuarenta años son propensos a mostrar complicaciones que limitan intensamente la función respiratoria.

Resume

Résection bilatérale dans le traitement de la tuberculose pulmonaire

La résection bilatérale a été préconisée chez 60 malades du "West Tennessee Tuberculosis Hospital." Le programme d'intervention bilatérale a été réalisé chez 33 malades il n'a pas été réalisé dans sa totalité chez 27 malades. Une analyse des résultats ultérieurs chez ces malades et une étude comparative des deux groupe montrent qu'en général les résultats obtenus ont été satisfaisants. Il existe une similitude qualitative dans les réactions pathologiques des deux poumons chez un même individu, de telle sorte que les signes anatomo-pathologiques constatés à la première intervention peuvent servir à évaluer la nécessité d'une intervention contro-latérale.

La résection bilatérale est bien tolérée par les malades du groupe le plus jeune, alors que les malades âgés de plus de 40 ans ont tendance à présenter des complications qui limitent considérablement la fonction respiratoire.

MYCOBACTERIUM ULCERANS INFECTIONS[1]

Response to Chemotherapy in Mice

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INTRODUCTION

Mycobacterium ulcerans, first described by MacCallum, Tolhurst, Buckle, and Sissons (1) in 1948, has several distinctions. Although pathogenic for man, recorded recognition of the disease with which this organism is associated has been limited to Australia (1), the Belgian Congo (2), and to one case in Mexico (3). The organism, which is strongly acid fast and alcohol fast, is fastidious in its temperature requirements for multiplication, growing best at 33°C. and not at 37°C. In man the infection has a marked predilection for the integument, in which the disease is characterized by deep indolent cutaneous ulcers and has a protracted course. In the original cases of MacCallum and associates, successful treatment consisted of wide excision of the involved area and skin grafting. Microscopically, the tissue reactions in the infected human being are quite dissimilar to those of tuberculosis or leprosy. The lesions in man, which are granulomatous, usually show congestion, edema, and evidence of secondary pyogenic infection superimposed on a more specific cellular response. The latter consists of macrophages and a scattering of lymphocytes and plasma cells. Acid-fast bacillary cells in variable numbers are present.

Experimentally, pathogenicity of *M. ulcerans* is best demonstrated in rats (1), mice (4, 5), and phalangers (6). Guinea pigs and chickens have little if any susceptibility to this organism, while rabbits are susceptible to a limited degree (1).

The striking deterrent effects *in vivo* of several antimicrobial agents against *Mycobacterium tuberculosis hominis* and *bovis* and the favorable action of certain substances, particularly the sulfones, against infections with *Mycobacterium leprae* (7) and *Mycobacterium lepraemurium* (8) raised speculation regarding the vulnerability to chemotherapy of infections induced experimentally with *M. ulcerans*. To obtain information regarding the response to chemotherapy of infections induced by *M. ulcerans*, the following limited study was done.

METHODS

Forty adult male white mice, Strain *C*, each weighing approximately 20 gm., were each inoculated intravenously by way of the tail vein with *M. ulcerans*, Harvey strain.[2] The

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[1] From the Section of Pathologic Anatomy and Section of Bacteriology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota. The Mayo Foundation, Rochester, Minnesota, is a part of the Graduate School of the University of Minnesota.

[2] This culture was obtained in 1950 from Mr. Glen Buckle, Bacteriology Department, Alfred Hospital, Prahran, Victoria, Australia. The subculture used had been incubated at 33°C. for approximately two months and represented the sixteenth successive subculture from the growth received originally from Mr. Buckle.

TABLE 1

MORTALITY OF TREATED AND UNTREATED GROUPS OF MICE INOCULATED IN A VEIN OF THE TAIL WITH M. ULCERANS

Groups	Animals	Died	Survivors*
Controls (not treated)	10	7	3
Treated: streptomycin, 3 mg. subcutaneously daily	10	4†	6
Treated: diaminodiphenylsulfone, 0.1 per cent in diet	10	3	7
Treated: isoniazid, 0.02 per cent in diet	10	5‡	5

* Killed after 116 days.

† One killed accidentally.

‡ All but 2 animals lived longer than 90 days.

inoculum represented a suspension in sterile water of *M. ulcerans* with a density comparable to tube 0.5 of the McFarland nephelometer. Each animal received 0.1 ml. of the suspension. The 40 animals were separated into four groups of 10 mice, and each group occupied one cage. The diet consisted of finely ground Fox Chow (Purina Mills). Fresh diet was given once daily. The four groups were designated respectively as follows: *Group 1*, untreated controls; *Group 2*, streptomycin-treated; *Group 3*, 4,4′-diaminodiphenylsulfone-treated; and *Group 4*, isoniazid-treated. Starting on the day of inoculation and continuing daily thereafter for the duration of the experiment, the therapeutic regimen was as follows: streptomycin given subcutaneously, 3 mg. once daily; diaminodiphenylsulfone, 0.1 per cent in the diet; isoniazid, 0.02 per cent in the diet.[3]

The experiment was terminated 116 days after the animals had been inoculated. At the time of necropsy, material from the following was preserved for subsequent microscopic examination: lungs, liver, spleen, kidneys, testes, and tail. In a few instances tissues including spleen and substernal lymph nodes were obtained for attempts to isolate *M. ulcerans* in culture.

RESULTS

Mortality: After 116 days, when the experiment was terminated, a total of 19 of the 40 animals had died. The mortality in the respective groups is summarized in table 1. The greater number of deaths, 12, occurred among the untreated control animals and in the group that received isoniazid. Conversely, the mortality in the groups that received streptomycin and diaminodiphenylsulfone, respectively, suggested that these antimicrobials had exerted a favorable influence on the mortality rate of the animals which received these drugs. During the period of observation 4 animals in one and 3 in the other of these latter groups died. The 4 deaths among those treated with streptomycin occurred 25, 39, 88, and 115 days, respectively, after inoculation. The cause of death of 3 of the mice is unknown, and one was killed accidentally. The 3 animals recorded as having died in the group that received diaminodiphenylsulfone died on the first, the third, and the fifth days, respectively, after the experiment was started. The results in these animals were considered experimental failures.

[3] It was estimated that each mouse consumed an average of 3 gm. of diet daily. The intake of diaminodiphenylsulfone was, therefore, approximately 3 mg. per day or 150 mg. per kg. of body weight. The intake of isoniazid was approximately 0.6 mg. per day or 30 mg. per kg. of body weight.

The days of survival of the 5 animals which died in the group that received isoniazid were as follows: 88, 88, 109, 110, and 112. Deaths of the animals in the control or untreated group which died before the end of the experiment were recorded in days as follows: 38, 76, 80, 83, 83, 88, and 101.

On the basis of survival alone, the data reveal that the groups that received antimicrobial therapy had a smaller percentage of deaths than the group that received no therapy. The ratios of the dead to the survivors in the three groups that were treated were 4 to 6, 3 to 7, and 5 to 5, respectively. Among the controls the ratio was 7 to 3. It appears, therefore, that in the groups that were treated the mortality was influenced favorably by therapy.

Recoverability of organisms: Unfortunately, circumstances developed after the experiment was under way that made it difficult, if not impossible, to attempt, at the time of necropsy, the isolation by culture of *M. ulcerans* from tissues of the inoculated animals. Thus, a regrettable void exists in the information that it was originally hoped to obtain. The tissues from only a few of the animals were cultured. These included a substernal lymph node from an untreated animal that died after 38 days. The results were positive at 33°C., but no growth occurred from comparable portions of the same material incubated at 37°C. Also cultured was the spleen from another of the animals in the control group that was killed 116 days after inoculation. Positive cultures were obtained from the splenic material incubated at 33°C. Cultures were attempted from the liver of a mouse in the group that received diaminodiphenylsulfone. This animal died 5 days after the experiment began. Cultures positive for *M. ulcerans* were obtained after incubation at 33°C. Finally, cultures positive for *M. ulcerans* were obtained at 33°C. incubation temperature from the spleen of one of the mice which received isoniazid and which was killed when the experiment was terminated. Incidentally, in the latter instance, a portion of the same spleen showed many large cells that resembled megalokaryocytes in the splenic pulp, but no acid-fast organisms were observed in the tissues.

This phase of the study is so meager as hardly to warrant mention. However, the positive cultures obtained indicate, as Fenner (5) has so adequately shown, that *M. ulcerans* introduced intravenously in the mouse may find extravascular lodgment in tissues other than the integument and remain viable for a considerable time without necessarily provoking demonstrable lesions.

Pathologic changes: Since the experiment was of relatively short duration, 116 days, certain effects of experimental infection with *M. ulcerans*, such as ascites and purulent orchitis, mentioned by others (1, 5) in animals observed for a much longer time, were not seen. Most of the inoculated animals seemed to tolerate the infection rather well, as indicated by the fact that among the 40 mice only 6 deaths occurred during the first 60 days and 3 of these occurred during the first 5 days. From the sixty-first through the ninetieth day, 8 animals died; and from the ninety-first to, but not including, the one hundred and sixteenth day, when the survivors were killed, 5 animals died.

The most impressive structural changes occurred in the tails of the animals in the control or untreated group and in the animals which received isoniazid.

FIG. 1. Three mice of the untreated control group. Photographed 81 days after intravenous inoculation with *M. ulcerans*. Note the swollen, eroded, nodular condition of the tails.

The changes in these animals were in marked contrast to those observed in the mice treated with streptomycin and those treated with diaminodiphenylsulfone. Some weeks after inoculation, the tails became scabrous and slightly to severely swollen from the proximal to the distal end. As the disease in the tails progressed, reddish brown, roughened excrescences developed, which in some instances involved the entire length of the appendage except the distal 1 to 2 cm. (figures 1 and 2). As the morbid process became more aggravated, the epidermis in many areas sloughed off, leaving a moist, raw, eroded surface followed often by the formation of crusts or rough scabs. Finally, if the infection was permitted to run its course, the more distal part of the tail became ischemic and atrophic. Eventually only the bony elements remained. In those instances in which the entire tail became greatly swollen, and in which the reaction to the infection involved practically all of the soft tissues, spontaneous amputation of all or part of the tail often occurred.

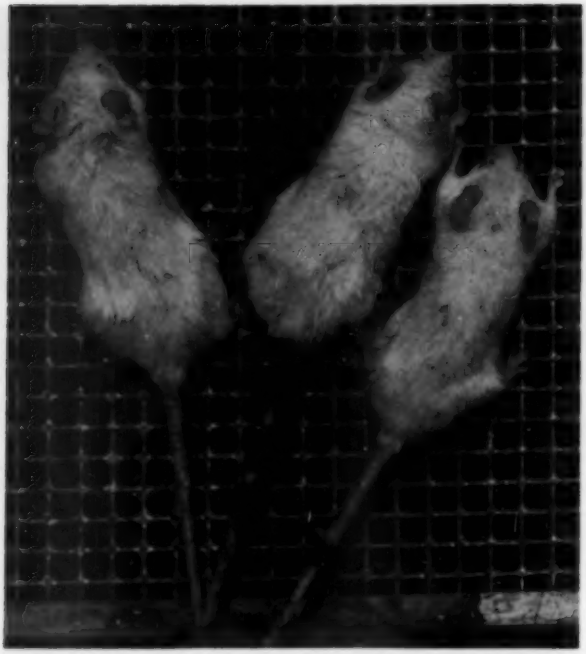

FIG. 2. Three mice of the group treated with isoniazid. Photographed 81 days after intravenous inoculation with *M. ulcerans*. The swollen, ulcerated condition of the tails is apparent.

Grossly, the structural changes, as a consequence of the infection in the respective groups of mice, differed greatly between the animals that were not treated and the animals that received isoniazid on the one hand and those that received streptomycin and diaminodiphenylsulfone, respectively, on the other. The lesions, which were limited with few exceptions to the tails, were strikingly evident in the untreated group and in the group treated with isoniazid, while the tails of those animals which had received the other two antimicrobials were essentially normal in appearance.

Microscopically, the tails of the animals that showed gross evidence of the infection revealed extensive and perhaps irreversible changes due to the severity of the reactive process (figure 3). From the periphery inward it was noted that the epidermis either was denuded or had become quite thin owing to the underlying inflammatory reaction. The corium was frequently thickened because of edema, with areas of necrosis extending to the underlying tendons and muscles. When the reaction was severe, marked congestion of the blood vessels was noted and many of the larger blood vessels in the subdermal area had become or were becoming thrombosed. The caudal muscles in the path of the extending infectious process were occasionally involved, and some degeneration and de-

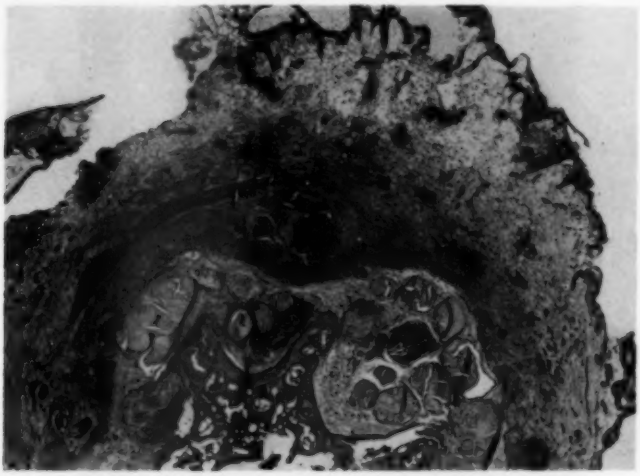

FIG. 3. Histopathologic features of a cross-section of the tail of a control or untreated mouse killed 116 days after intravenous inoculation with *M. ulcerans*. Most of the epidermis had sloughed off. The corium shows a diffuse granulomatous reaction, with much edema. The reactive process extends into the zone of muscles and tendons and to the periosteum. Accumulations of acid-fast bacillary rods were present, particularly in the subdermal areas. Involvement of the marrow was not observed. (×30)

struction of muscle occurred. This was characterized by vascularization, loss of muscle fibers, and the presence of a few lymphocytes. However, mass destruction of the muscle was rarely observed. The reaction to the infection was situated largely in the connective-tissue elements of the capsule or epimysium.

The tendinous components of the caudal appendage were likewise relatively resistant to the infection. The caudal tendons, which are quite conspicuous in the tail of the mouse, generally remained intact, although the fibrous sheaths of the tendons were usually involved in a reactive response. The reaction in the fibrous sheaths was of variable severity and was often striking. Marked edema, congestion of the blood vessels with occasional thrombosis, infiltration of lymphocytes, the appearance of histiocytic elements and granulomatous tissue, and necrosis were observed. Acid-fast bacillary cells were present, usually in tremendous numbers. In some instances in which the reaction was especially violent and extensive, the connective tissue comprising the peritenoneum was much thickened and revealed the same reactive phenomenon as the tendon sheaths mentioned previously.

Of unusual interest was the finding, in the tails of a few animals, of mycobacteria within the substance of nerves, which in the mouse occur rather prominently among the tendons. A few to several of the acid-fast bacilli were seen in the nerve tissue in and around the myelin sheaths and among the fibrocytic elements adjacent to small blood vessels. In no instance was there evidence of

FIG. 4. Histopathologic features of a cross-section of the tail of a mouse treated with isoniazid and killed 116 days after intravenous inoculation with *M. ulcerans*. Some denudation of the epidermis and marked thickening of the cutaneous and subcutaneous areas may be observed. The reactive process extends into the connective tissue surrounding the muscles and tendons, with destruction of the former. Note the absence of normal myeloid tissue. Acid-fast bacilli were exceedingly numerous among the inflammatory elements of the subcutis and among the reactive components surrounding the muscles and tendons. Many similar bacteria were present in the marrow space. (×16)

any cellular or other reactions to the presence of the microorganism within the nerves.[4]

The involvement of the myeloid substance of the caudal vertebrae by the infectious process was frequently striking. Although the soft tissues surrounding the vertebrae were frequently the site of an active, progressive, and destructive phenomenon, the osseous tissues of the vertebrae successfully resisted the advancing onslaught. However, in those mice in the untreated or control group and those that received isoniazid, the myeloid tissue was invariably affected. The degree of involvement varied, but in most instances in which the animals

[4] In the interpretation of the finding of acid-fast bacilli in the peripheral nerves of mice inoculated with *M. ulcerans*, certain difficulties and uncertainties must be considered. Foremost among the questions that arise is: Are the bacteria observed in nerves artifacts, due to bacilli being dislodged during the preparation of the tissue section from areas nearby where the bacteria are numerous, or do they represent dissemination into the nerves during the pathogenesis of the infection?

FIG. 5. Three mice of the group treated with diaminodiphenylsulfone. Photographed 81 days after intravenous inoculation with *M. ulcerans*. The smooth, intact condition of the integument of the tail is in marked contrast to the appearance of the tails in figures 1 and 2.

had been inoculated 116 days before necrospy a profound change in the marrow space had taken place (figure 4). In many instances the myeloid elements had lost their identifying characteristics. An alveolar-like stroma was usually discernible, but the fat components had disappeared. The cells that normally occupy the connective tissue between the fat cells were few and had apparently lost their capacity for hemopoiesis. As a matter of fact, in most instances the marrow tissue had been replaced by acid-fast bacillary cells, which were often present in dense accumulations. Cells containing the bacteria appeared to be in a degenerative state, although evidence of definite necrosis was not apparent. Signs of a granulomatous response in the marrow were minimal.

In the marrow the infection stopped at the epiphyses, and in no instance were the intervertebral disks involved.

Despite the impressive amount of disease in the tissues of the caudal ap-

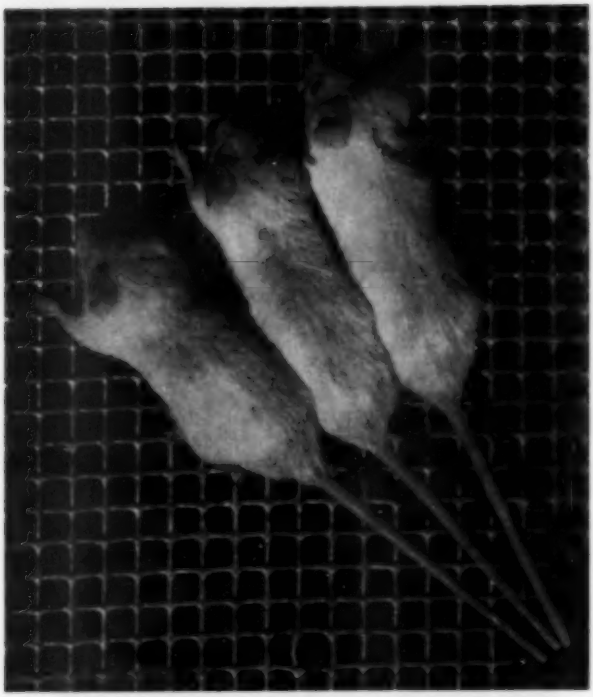

FIG. 6. Three mice of the group treated with streptomycin. Photographed 81 days after intravenous inoculation with *M. ulcerans*. In contrast to the animals shown in figures 1 and 2, the tails of the mice in this group appear essentially normal.

pendages of the mice in the control group and in the group that received isoniazid, the other tissues examined microscopically, i.e., the spleen, lungs, kidneys, liver, and testes, were devoid of signs of infection with *M. ulcerans*. Except for an instance of non-mycobacterial pneumonia in one mouse and an occasional instance of purulent adenitis, in one of which mycobacteria were obtained by culture, no significant structural changes were observed except those that occurred in the tail.

The histopathologic characteristics of the tails of the groups of mice that received streptomycin and diaminodiphenylsulfone, respectively, were generally in conformity with the impressions obtained from the gross appearances of these appendages 81 days after the experiment was started (figures 5 and 6). Of the 10 mice in the group that received streptomycin, tissues for microscopic study were available from 7. In none of the tissues examined, including the tail, from these 7 mice were lesions found that were related to the experimental inoculation. The only acid-fast bacilli found occurred in the substance of one of the larger caudal nerves in an animal which died after 115 days. This finding was

probably not an artifact, since similar organisms in the same anatomic situation were present in serial sections of the same material.

From 7 of the 10 mice that received diaminodiphenylsulfone, tissues from the tail and other organs, including the testes, liver, spleen, lungs, and kidneys, were examined microscopically. In none of the material examined were there any detectable signs of an infectious process, and no acid-fast bacilli were observed in any of the tissues.

The results obtained as a consequence of the comparative study of material from the four groups of mice in this experiment indicate without equivocation that the animals that received streptomycin and diaminodiphenylsulfone, respectively, were, at the time of necropsy, essentially free of any demonstrable anatomic signs of infection. This was so despite the fact that the animals had received intravenously an infective inoculum which in untreated mice and in mice treated with isoniazid produced in practically every instance a progressive, destructive, and irreversible disease, frequently resulting in the spontaneous amputation of a portion or of all of the tail.

Discussion

The experiment described exemplifies the possibility that antimicrobial drugs effective *in vivo* against one species of mycobacteria may be capable of exerting an inhibitory action against other and markedly different species of the same genus. Evidence exists to support the conclusion that the degree of specificity of drugs varies markedly with the different mycobacteria. Of the several substances known to be effective against tuberculous infections, diaminodiphenylsulfone probably has the broadest spectrum of antimycobacterial action, being also effective against human leprosy, murine leprosy, and infections produced by *M. ulcerans*. The therapeutic potential of this sulfone is worthy of further study, particularly in certain mycobacterial diseases of animals such as paratuberculosis (Johne's disease) (9) and the so-called "skin" or tuberculoid lesions of cattle (10).[5]

It may be significant that antimicrobial substances effective *in vivo* against an infection due to a given species of mycobacteria may be effective against other mycobacteria. This emphasizes the importance of submitting any new substance that is capable of a deterrent action against one mycobacterial infection to experimental trials with as many different forms of pathogenic mycobacteria as possible. Many of the mycobacterial infections remain intractable to chemotherapy, and new and more potent substances could well be useful and important additions to the present list of therapeutic agents.

[5] Larsen and Vardaman (11) reported *Mycobacterium paratuberculosis in vitro* to be relatively insensitive to diaminodiphenylsulfone, but considerably more sensitive to viomycin. The same writers treated, with diaminodiphenylsulfone given by mouth daily, one bovine animal with Johne's disease in an advanced stage. The daily dose varied from 10 to 35 mg. per pound of body weight. Treatment was continued for 4 weeks. Some initial improvement was noted, but exacerbation of symptoms occurred and the animal was considered incurable and was killed.

The experiment recounted in the present study, while of limited scope, did yield results that are of some significance in revealing the impressive ability of streptomycin and of diaminodiphenylsulfone to interfere with the natural progression of infections due to *M. ulcerans*. The results also demonstrated the inferiority of the antagonistic action against *M. ulcerans* of isoniazid compared with the other two substances. About the only favorable effect noted following treatment with isoniazid was a lessening of the tempo of pathogenesis. Despite the fact that the mice in this group received isoniazid continuously from the day on which the animals were inoculated, the infection did become established and progressed without interruption during the period of observation. It is true that the mice that received isoniazid appeared to tolerate the infection somewhat better than did the untreated controls, but other objective signs of a beneficial effect were lacking.[6]

The effectiveness of streptomycin against experimental infections of mice with *M. ulcerans* has also been reported by Leach and Fenner (4). The animals were inoculated into the foot pad, and streptomycin was given intraperitoneally in doses of 1.5 mg. six times weekly. Three treatment regimens were followed. In one group treatment was started on the day of inoculation; in the second group treatment was delayed until signs of the infection became apparent at the site of inoculation; and in the third group treatment was withheld until disease in the foot pad progressed to an advanced stage. The most effective results were noted in the mice in which treatment began at the time of inoculation or at the time when lesions were first observed. In the mice in which the disease in the foot had progressed to an advanced stage before treatment was started, streptomycin was of no avail.

According to Penington (12), of Melbourne, Australia, a case of infection with *M. ulcerans* in a human being responded satisfactorily to treatment with streptomycin and Promin® jelly. The dose regimen of streptomycin was 0.5 gm. daily. Lavalle Aguilar, Marquez Iturribarria, and Middlebrook (3) described a case of severe infection of the hand of a Mexican farmer by *M. ulcerans* which was treated with diaminodiphenylsulfone.[7] The dosage was 100 mg. daily for 15 days, followed by 200 mg. daily, which was continued with short periods of interruption for a total of approximately 8 months. The response to treatment was slow but definite, and at the time the case was reported signs of activity had almost completely disappeared. No information is available concerning the use of isoniazid in clinical infections with this organism.

The character of the experiment which is the subject of this report limited the scope of the information that might have been obtained regarding the potential effectiveness of the two substances that proved definitely antagonistic to the

[6] Leach and Fenner (4) tested *in vitro* the susceptibility of four strains of *M. ulcerans* to isoniazid, PAS, and thiosemicarbazone, respectively. In concentrations sufficient to prevent growth of the H37Rv strain of tubercle bacilli, the drugs mentioned had little if any inhibitory effect on *M. ulcerans*. The highest concentration used was 10 mg. per ml. Conversely, *M. ulcerans* was found to be highly susceptible *in vitro* to streptomycin.

[7] This was the first case of human infection by *M. ulcerans* reported from the Western Hemisphere.

infection. Since medication was started on the same day on which the animals were inoculated, and before any appreciable reaction on the part of the host was present, the character of the experiment was more prophylactic than truly therapeutic. Another experiment is contemplated using mice inoculated intravenously and permitting the infection to become well established before antimicrobial treatment is started. The relatively slow tempo of the progression of experimental infection with *M. ulcerans* suggests the advisability of continuing treatment for a much longer period than was the case in the present experiment. The impressive multiplication of the organisms and the severe inflammatory response of the host in sites of predilection are formidable obstacles to a quick and permanent elimination of *M. ulcerans* from the areas of the morbid reaction by any therapeutic substance presently known.

Summary

To determine the effect of certain antimicrobials on the development and progression of infections in mice due to *Mycobacterium ulcerans*, an experiment was done involving four groups of 10 male white mice each. All mice were inoculated intravenously via the tail vein. One group of 10 mice received no medication. The other three groups received on the day of inoculation streptomycin parenterally and 4,4′-diaminodiphenylsulfone and isoniazid, respectively, in the diet. Administration of the drugs continued daily for the duration of the experiment, which was terminated after 116 days. The effectiveness of the different treatment regimens was determined principally by microscopic examination of the tails, which were the site of predilection for the infectious process. The lungs, liver, spleen, testes, and kidneys were likewise examined. Also noted were the comparative survival times of the mice in the different groups.

The data obtained indicated quite definitely that, under the conditions of the experiment, streptomycin and 4,4′-diaminodiphenylsulfone were markedly effective in inhibiting the pathogenesis of the infection. The action of isoniazid was less favorable. While the latter substance appeared to have extended the lives of many of the animals which received this drug, the severity of the infection in the animals treated with isoniazid was comparable with that observed in the untreated controls. The specific localization of the lesions following the inoculation of *M. ulcerans* illustrates the dependence for the multiplication of this organism *in vivo* on the favorable temperature of the environment.

Sumario

Infecciones por Mycobacterium Ulcerans: Respuesta a la Quimioterapia en los Ratones

A fin de determinar el efecto de ciertas drogas antimicrobianas sobre la aparición y el avance de infecciones murinas debidas al *Mycobacterium ulcerans*, se llevó a cabo un experimento que comprendió cuatro grupos de 10 ratones blancos machos cada uno. A todos se les inoculó intravenosamente por la vena del rabo. Un grupo de 10 ratones no recibió medicación. Los otros tres grupos recibieron el día de la inoculación estreptomicina parentéricamente y 4,4′-diaminodifenilsulfona e isoniacida, respectivamente, en la alimentación. La administración de estas drogas continuó a diario mientras duró el experimento, que terminó al cabo de 116 días. La eficacia de los diversos regímenes terapéuticos se determinó princi-

palmente por el examen microscópico de los rabos, que fueron el sitio predilecto del proceso infeccioso. Se examinaron igualmente los pulmones, hígado, bazo, testículos y riñones. También se anotaron los tiempos comparativos de sobrevivencia de los ratones de los distintos grupos.

Los datos obtenidos indicaron bastante definitivamente que, en las condiciones del experimento, la estreptomicina y la 4,4′-diaminodifenilsulfona eran notablemente eficaces para inhibir la patogenia de la infección. El efecto de la isoniacida fué menos favorable. Aunque esta última substancia pareció prolongar la vida de muchos de los animales que la recibieron, la gravedad de la infección en los animales tratados con ella fué comparable a la observada en los testigos no tratados. La localización específica de las lesiones consecutivas a la inoculación de *M. ulcerans* ejemplifica la forma en que depende la multiplicación *in vivo* de este microbio de la temperatura favorable del medio ambiente.

Resume

Infections à Mycobacterium ulcerans: Réponse à la chimiothérapie chez la souris

Pour déterminer l'effet de certains agents antibactériens sur le développement et la progression des infections à *M. ulcerans* chez les souris, il a été procédé à une expérimentation portant sur quatre groupes de 10 souris blanches mâles chacun. Toutes les souris furent inoculées par voie intraveineuse par la veine caudale. Un groupe de 10 souris est resté sans médication. Le jour de l'inoculation, les trois autres groupes reçurent de la streptomycine par voie parentérale ainsi que de la 4,4′-diaminodiphénylsulfone et de l'isoniazide, respectivement, dans la ration alimentaire. L'administration quotidienne de ces drogues fut continuée pendant la durée de l'expérimentation qui prit fin au bout de 116 jours. L'efficacité des différentes thérapeutiques fut principalement déterminée par l'examen histologique des queues, qui constituaient le siège de prédilection du processus infectieux. Les poumons, le foie, la rate, les testicules et les reins furent examinés de la même manière. Les temps de survie des souris furent également enregistrés dans les différents groupes.

Les données obtenues démontraient de façon précise que, dans les conditions expérimentales, la streptomycine et la 4,4′-diaminodiphénylsulfone exerçaient un pouvoir inhibiteur marqué sur la pathogénèse de l'infection. L'action de l'isoniazide était moins satisfaisante. Tandis que cette dernière substance paraissait prolonger la vie de nombreux animaux auxquels elle était administrée, la sévérité de l'infection chez les animaux traités par l'isoniazide était comparable à celle des témoins non traités. La localisation spécifique des lésions succédant à l'inoculation de *M. ulcerans* illustre le fait que la reproduction *in vivo* de ce germe est subordonnée à l'influence d'une température ambiante favorable.

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THE ENUMERATION OF NONPATHOGENIC VIABLE TUBERCLE BACILLI FROM THE ORGANS OF MICE[1, 2]

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INTRODUCTION

It has been shown by Fenner (1) and Fenner, Martin, and Pierce (2) that the number of viable particles of tubercle bacilli found in cultures grown in a medium containing a dispersing agent, or found in homogenized tissues of infected animals, can be enumerated with a reasonable degree of accuracy by the drop plate counting technique of Miles and Misra (3).

It is important to keep in mind, however, that the validity of the results of experiments designed to enumerate viable organisms in the tissues of animals will depend not only on the reliability of the plate counting technique employed but, in large measure, will depend also upon the degree of variation encountered in microbial content between tissues or organs of different animals. A sufficient number of animals must be sacrificed at each interval, and the microbial content of the organs of each measured, so that the limits of variation between animals may be calculated. Although Fenner, Martin, and Pierce (2) foresaw the large error which might be introduced by this type of variation, they made no measurement of it.

Several investigators, Pierce, Dubos, and Schaefer (4), Mackaness, Smith, and Wells (5), Dubos (6, 7), and Bloch and Segal (8), have attempted to follow the survival of a variety of strains of mycobacteria in normal mice using the drop plate counting technique. Kanai and Yanagisawa (9) and Kanai, Katsuyama, and Yanagisawa (10) also have reported using this technique for the enumeration of tubercle bacilli from the organs of intravenously injected guinea pigs. Pierce and associates (4) found no evidence of multiplication of the H37Ra strain of *M. tuberculosis* var. *hominis* in the spleens of mice when they used the drop plate counting method for enumeration of the number of viable particles, whereas Mackaness and co-workers (5) noted an appreciable increase in the number of viable particles of the same strain in the spleens of mice. Also, on the basis of their findings, Pierce and her colleagues (4) have divided mycobacteria into three categories. "Avirulent" mycobacteria were those found to be unable to multiply *in vivo*, although they could survive many weeks in the tissues of mice. "Attenuated" mycobacteria were those forms which underwent multiplication

[1] From the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

[2] This investigation was supported in part by research grants from the Illinois Tuberculosis Association and The Tuberculosis Institute of Chicago.

[3] Fellow of the American Trudeau Society, medical section of the National Tuberculosis Association.

in vivo, but could not give rise to progressive disease. "Virulent" mycobacteria were those capable of establishing active progressive infection in mice even when small infective doses were used.

None of these investigators, however, has considered adequately the error that will be introduced by the variation which will occur in microbial population between the organs of different animals.

It is the purpose of the present paper to furnish data which will serve not only to define the limits of reliability of the drop plate technique for the determination of the number of tubercle bacilli in homogenates prepared from the organs of mice, but also to show the range of variation in microbial content between the same organs of different mice. This will make it possible to obtain a valid picture of the fate of cells of *Mycobacterium tuberculosis* in the organs of mice.

Methods

Mycobacterial cultures: The H37Ra,[4] R1Rv,[4] and BCG-4[5] strains were employed in this study. Three-week-old surface pellicle cultures of the H37Ra and R1Rv strains were harvested from modified Proskauer and Beck medium, and cell suspensions were prepared and standardized using methods previously described in detail (11). Fresh cultures of the BCG-4 strain from Sauton's medium were obtained, and suspensions were prepared and standardized by the same methods as those employed for the H37Ra and R1Rv strains. Following standardization the suspensions were diluted so that each 0.4 ml. contained 1.0 mg. wet weight of cells. Four tenfold serial dilutions of the H37Ra and BCG strains also were prepared (0.1 mg., 0.01 mg., 0.001 mg., and 0.0001 mg.). The number of viable particles of tubercle bacilli in these suspensions was determined by a modification of Fenner's modification (1) of the drop plate counting technique of Miles and Misra (3). This technique will be described below. Ziehl-Neelsen stained preparations of suspensions of cultures used for injection and tissue homogenates used for plating were examined microscopically and were found to consist predominantly of single acid-fast cells. Only occasional clumps of three to ten organisms could be seen.

Inoculum: Mice were injected intravenously with 0.4 ml. of the suspension of the mycobacterial strain to be studied. The number of viable particles of tubercle bacilli contained in the 1.0 mg. inoculum was approximately forty million of the H37Ra and R1Rv strains and approximately six million of the BCG strain. The four serial dilutions of the H37Ra and BCG strains were found to contain proportionately smaller numbers of viable particles of tubercle bacilli (H37Ra: approximately 4,000,000, 400,000, 40,000, and 4,000 particles in 0.4 ml., respectively; BCG: approximately 600,000, 60,000, 6,000, and 600 particles in 0.4 ml., respectively).

Mice: Two strains of mice were employed: The "Strong A" strain, which has been reported to be fairly resistant to infection with tubercle bacilli (12), and the "C57" strain, generally regarded as highly susceptible to infection with tubercle bacilli (12). For each experiment 40 female mice were injected intravenously.

Enumeration technique: At appropriate intervals following injection, 5 mice were killed by crushing their skulls. The lungs, livers, and spleens were removed aseptically and weighed. The organs were then homogenized individually by grinding for two minutes at

[4] Obtained from Dr. William Steenken, Jr., Trudeau Laboratories, Trudeau, New York.

[5] BCG strain No. 4 is a highly immunogenic strain obtained from Dr. Sol Roy Rosenthal, Tice Clinic, Chicago, Illinois.

1,100 r.p.m. with sterile Teflon® grinders in matching tubes. The tissue was then diluted to a volume of 10.0 ml. with 0.01 molar phosphate buffer solution, pH 7.0, and the homogenization procedure was continued for two minutes. Appropriate serial dilutions of this final homogenate were made in phosphate buffer solution containing 1.0 per cent of fraction V bovine albumin.

Modified Proskauer and Beck medium containing 1.5 per cent agar and 5.0 per cent sterile bovine serum was used as the plating medium. Using 0.1 ml. pipettes, ten 0.02 ml. volumes of each dilution of each homogenate were placed approximately 2 cm. apart upon the surface of each of two plates, making five replicates per plate. The plates were incubated at 37°C. in closed containers to prevent drying of the medium. At the end of twenty-one days the number of colonies contained in each area was counted and recorded. Computations were made only from those data obtained from plates that showed between ten and forty colonies.

Originally, samplings were made with the BCG-4 strain from the mouse population every day for the first seven days. Upon finding that no significant deviations from the observed pattern occurred during this period, representative samplings were made thereafter on the third day after injection and at appropriate intervals for forty-eight days. The confidence limits of the mean number of organisms recovered per organ and the probability of significant change in means were determined for a probability of 0.05. The coefficient of vari-

TABLE 1

VIABLE PARTICLES OF THE H37Ra STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF "STRONG A" MICE FOLLOWING INTRAVENOUS INJECTION*

Days After Injection	Mean Number of Viable Particles Recovered per Organ	95 Per Cent Confidence Limits†	Significant Change‡	Coefficient of Variation for Replicate Organs	Coefficient of Variation for Replicate Platings	Dilution Error
						per cent
Lung						
3	361,250	±254,465		57.7	3.6	5
10	755,100	±248,200		27.7	4.3	5
19	1,831,000	±794,654	+	35.5	4.2	5
26	244,100	±87,792	+	31.6	6.4	5
33	77,350	±35,340	+	36.9	1.4	5
47	116,350	±34,348		23.8	7.4	5
Liver						
3	20,400,000	±4,979,520		16.2	5.6	5
10	8,840,000	±3,069,000	+	28.0	7.6	5
19	10,150,000	±1,271,000		10.1	6.0	5
26	354,200	±76,012	+	17.3	4.5	5
33	185,250	±60,140	+	26.2	2.9	5
47	176,000	±55,428		25.4	6.1	5
Spleen						
3	2,077,000	±190,588		7.4	6.5	5
10	1,890,000	±489,800		20.9	10.4	5
19	1,603,000	±427,304		21.5	5.4	5
26	82,350	±29,407	+	28.8	8.1	5
33	23,400	±20,708	+	71.6	2.4	5
47	8,975	±24,800		22.6	6.4	5

* 1 mg.–0.4 ml.—40,660,000 viable organisms SD ± 6,280,000.

† Confidence limits derived from "t" × (Standard Error).

‡ Indicates significant change in mean number of viable particles recovered per organ since the previous determination—derived from "t" values (P = 0.05).

ation for replicate organs was calculated each time. However, only the largest coefficient of variation for replicate platings of each organ of the five organs plated on a single day was recorded. The dilution error was determined from the tables of Jennison and Wadsworth (13).

Results

In tables 1–6 and in the corresponding figures 1–6 are summarized the results of the experiments in which the fate of the 1.0 mg. dose of H37Ra, R1Rv, and BCG strains of *M. tuberculosis* in the organs of two strains of mice, "Strong A" and "C57," was followed by the drop plate counting technique.

The reliability of the plate count technique for the enumeration of all three strains of mycobacteria, as determined by the coefficient of variation for replicate platings and the dilution error, was such that the mean of the coefficients of variation for replicate platings was 4.6 per cent with a range of 1.4 to 10.4 per cent. The dilution error as determined from the figures of Jennison and Wadsworth (13) was 5 per cent.

The variation between mice was such that the mean for the coefficients of variation for replicate organs was 26.1 per cent with a range of 7.4 to 71.6 per cent. The confidence limits were determined for a probability of 0.05. With this

TABLE 2

VIABLE PARTICLES OF THE H37RA STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF "C57" STRAIN MICE FOLLOWING INTRAVENOUS INJECTION*

Days After Injection	Mean Number of Viable Particles Recovered per Organ	95 Per Cent Confidence Limits†	Significant Change‡	Coefficient of Variation for Replicate Organs	Coefficient of Variation for Replicate Platings	Dilution Error
						per cent
Lung						
3	410,100	±215,105		42.3	4.6	5
9	906,000	±229,181		20.4	4.8	5
18	1,966,000	±672,843	+	27.6	3.7	5
25	267,000	±107,463	+	31.4	5.2	5
32	81,000	±26,717	+	26.6	4.8	5
Liver						
3	17,876,000	±4,477,580		20.2	3.2	5
9	12,965,000	±2,700,868		16.8	5.8	5
18	8,230,000	±1,877,756	+	18.4	4.8	5
25	610,000	±193,638	+	25.6	4.5	5
32	104,000	±30,176	+	23.4	3.0	5
Spleen						
3	1,793,000	±720,355		32.4	5.4	5
9	1,687,000	±753,076		36.0	4.0	5
18	1,536,000	±662,814		34.8	3.2	5
25	102,000	±26,307	+	20.8	4.6	5
32	17,100	±3,943	+	18.6	4.2	5

* 1 mg.–0.4 ml.—40,352,000 viable organisms SD ±6,268,000.

† Confidence limits derived from "t" × (Standard Error).

‡ Indicates significant change in mean number of viable particles recovered per organ since the previous determination—derived from "t" values (P = 0.05).

TABLE 3

VIABLE PARTICLES OF THE BCG STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF "STRONG A" MICE FOLLOWING INTRAVENOUS INJECTION*

Days After Injection	Mean Number of Viable Particles Recovered per Organ	95 Per Cent Confidence Limits†	Significant Change‡	Coefficient of Variation for Replicate Organs	Coefficient of Variation for Replicate Platings	Dilution Error
						per cent
Lung						
3	83,500	±42,656		41.2	6.1	5
7	87,000	±31,100		31.6	4.5	5
17	183,000	±53,816	+	23.7	5.2	5
26	49,200	±1,736	+	28.0	4.0	5
33	14,100	±4,464	+	25.6	4.8	5
Liver						
3	5,250,000	±1,386,320		21.3	5.8	5
7	3,080,000	±771,478	+	20.2	4.6	5
17	1,330,000	±276,520	+	16.8	3.2	5
26	116,500	±41,540	+	28.8	5.1	5
33	5,650	±1,426	+	20.6	4.6	5
Spleen						
3	695,000	±265,360		30.8	4.7	5
7	437,000	±147,560		27.3	3.9	5
17	402,000	±127,110		25.5	4.6	5
26	6,200	±2,000	+	30.5	4.7	5
33	None					

* 1 mg.-0.4 ml.—6,780,000 viable organisms SD ±237,000.

† Confidence limits derived from "t" × (Standard Error).

‡ Indicates significant change in mean number of viable particles recovered per organ since the previous determination—derived from "t" values (P = 0.05).

TABLE 4

VIABLE PARTICLES OF THE BCG STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF "C57" STRAIN MICE FOLLOWING INTRAVENOUS INJECTION*

Days After Injection	Mean Number of Viable Particles Recovered per Organ	95 Per Cent Confidence Limits†	Significant Change‡	Coefficient of Variation for Replicate Organs	Coefficient of Variation for Replicate Platings	Dilution Error
						per cent
Lung						
3	86,500	±35,181		32.8	4.2	5
9	97,200	±29,649		24.6	4.8	5
18	197,000	±64,000	+	26.2	5.0	5
25	16,000	±6,071	+	30.6	5.2	5
32	None					
Liver						
3	6,350,000	±976,376		12.4	3.6	5
9	3,918,000	±816,930	+	16.8	3.8	5
18	1,487,000	±346,649	+	18.8	3.2	5
25	153,100	±57,332	+	30.2	4.8	5
32	6,000	±2,574	+	34.6	4.0	5
Spleen						
3	586,000	±199,099		27.4	5.0	
9	580,000	±192,746		26.8	3.2	
18	410,000	±125,066		24.6	5.6	
25	6,000	±2,276	+	30.6	4.8	5
32	None					

* 1 mg.-0.4 ml.—6,395,000 viable organisms SD ±268,000.

† Confidence limits derived from "t" × (Standard Error).

‡ Indicates significant change in mean number of viable particles recovered per organ since the previous determination—derived from "t" values (P = 0.05).

TABLE 5

VIABLE PARTICLES OF THE R1Rv STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF "STRONG A" MICE FOLLOWING INTRAVENOUS INJECTION*

Days After Injection	Mean Number of Viable Particles Recovered per Organ	95 Per Cent Confidence Limits†	Significant Change‡	Coefficient of Variation for Replicate Organs	Coefficient of Variation for Replicate Platings	Dilution Error
						per cent
Lung						
3	383,000	±106,382		22.4	4.5	5
10	760,000	±261,987		27.8	3.8	5
19	1,930,000	±689,241	+	28.8	3.9	5
26	251,000	±96,163	+	30.1	4.2	5
33	79,000	±40,359	+	41.2	5.0	5
47	78,000	±35,592		36.8	3.8	5
Liver						
3	18,150,000	±3,330,888		14.8	6.0	5
10	12,100,000	±2,805,748	+	18.7	4.8	5
19	6,951,000	±2,154,810	+	25.0	4.6	5
26	390,000	±137,342	+	28.4	2.0	5
33	133,000	±34,138	+	20.7	5.7	5
47	129,000	±34,391		21.5	4.6	5
Spleen						
3	1,978,000	±755,437		30.8	5.0	5
10	1,913,000	±683,170		28.8	4.8	5
19	1,812,000	±595,423		26.6	2.8	5
26	83,000	±21,407	+	20.8	3.6	5
33	27,000	±7,834	+	23.4	3.2	5
47	10,000	±2,070	+	16.7	3.6	5

* 1 mg.–0.4 ml.—40,868,000 viable organisms SD ±6,886,000.

† Confidence limits derived from "t" × (Standard Error).

‡ Indicates significant change in mean number of viable particles recovered per organ since the previous determination—derived from "t" values (P = 0.05).

assumed probability, one-third of the means of the number of viable particles recovered per organ was not significantly different from the mean number recovered at the time of the previous plating.

The enumeration of the H37Ra, BCG, and R1Rv strains in the organs of "Strong A" and "C57" strain mice showed that, in each case, the initial distribution of viable particles in the three organs studied was approximately 2 per cent in the lungs, 90 per cent in the liver, and 8 per cent in the spleen. Approximately 50 per cent of the injected H37Ra and R1Rv organisms and 90 per cent of the injected BCG organisms could be recovered three days after injection from the three organs studied. There was a slight but significant increase in the number of mycobacteria of all three strains in the lung during the first two weeks of the experiment. There was a rapid decrease in numbers in the liver, and no significant change in numbers in the spleen during this period. During the remainder of the experiment, there was a rapid decrease in the number of viable particles in the lung and the spleen and a continuation of the decrease in the liver. By the end of

TABLE 6

VIABLE PARTICLES OF THE R1Rv STRAIN OF MYCOBACTERIUM TUBERCULOSIS RECOVERED FROM ORGANS OF "C57" STRAIN MICE FOLLOWING INTRAVENOUS INJECTION*

Days After Injection	Mean Number of Viable Particles Recovered per Organ	95 Per Cent Confidence Limits†	Significant Change‡	Coefficient of Variation for Replicate Organs	Coefficient of Variation for Replicate Platings	Dilution Error
						per cent
Lung						
3	401,000	±154,144		31.0	5.4	5
10	783,000	±317,486		22.4	5.2	5
19	2,001,000	±511,135	+	20.6	4.0	5
26	291,000	±103,200	+	28.6	4.6	5
32	93,000	±35,518	+	30.8	3.8	5
48	87,000	±28,475		26.4	3.2	5
Liver						
3	22,100,000	±4,302,428		15.7	3.8	5
10	13,300,000	±3,364,368	+	20.4	3.6	5
19	7,100,000	±915,616	+	10.4	4.0	5
26	379,000	±122,189	+	26.0	5.2	5
32	140,000	±84,369	+	48.6	3.2	5
48	126,000	±47,184		30.2	3.4	5
Spleen						
3	2,203,000	±830,442		30.4	5.0	5
10	1,820,000	±595,795		26.4	3.4	5
19	1,710,000	±597,952		28.2	4.8	5
26	87,000	±9,277	+	8.6	4.8	5
32	21,400	±5,464	+	20.6	3.5	5
48	7,300	±2,760	+	30.5	3.2	5

* 1 mg.-0.4 ml.—40,796,000 viable organisms SD ±6,548,000.

† Confidence limits derived from "t" × (Standard Error).

‡ Indicates significant change in mean number of viable particles recovered per organ since the previous determination—derived from "t" values (P = 0.05).

approximately five weeks, less than 1 per cent of the original number of viable particles remained.

The enumeration of the number of viable particles of the 0.1 mg., 0.01 mg., and 0.001 mg. doses of H37Ra and BCG from the organs of "Strong A" mice revealed identical distributions of viable particles and parallel courses of increase and decrease in the number of viable particles when compared with the 1 mg. dose, although, of course, the mean numbers of viable particles recovered per organ were proportionately smaller than those determined for the 1 mg. dose. Some of the platings of the organs of mice injected with 0.01 mg. and 0.001 mg. suspensions of mycobacteria and all of the platings of the organs of the mice injected with 0.0001 mg. suspensions of mycobacteria revealed no viable particles during the forty-eight-day period. This occurred because the number of bacilli in the organ was less than the lower limit of sensitivity of the technique as employed (5,000 viable particles).

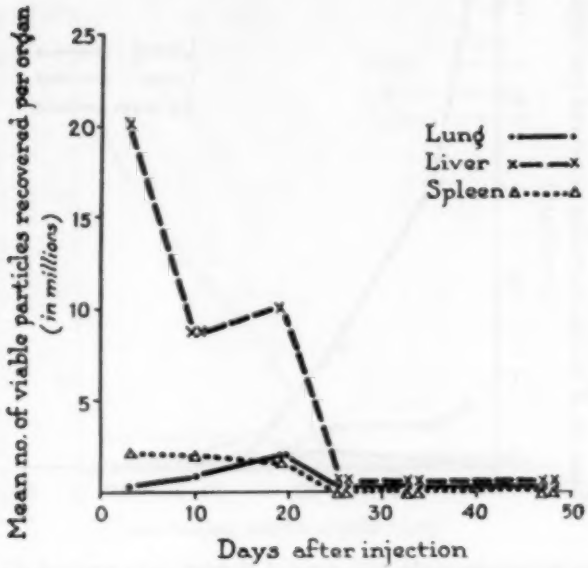

FIG. 1. Viable particles of the H37Ra strain of *M. tuberculosis* recovered from organs of "Strong A" mice (. ., ××, ΔΔ indicate significant change from previous determination).

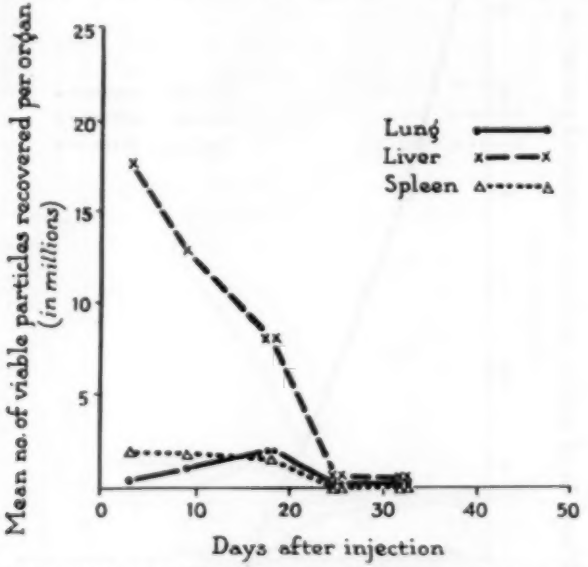

FIG. 2. Viable particles of the H37Ra strain of *M. tuberculosis* recovered from organs of "C57" mice (. ., ××, ΔΔ indicate significant change from previous determination).

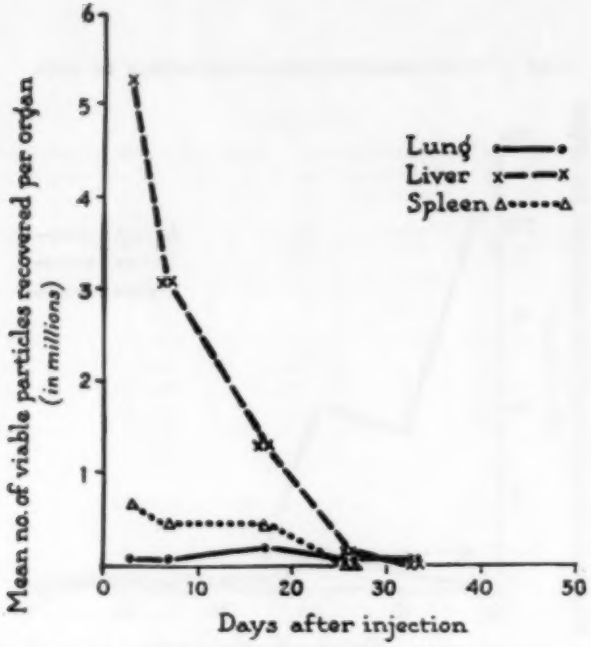

FIG. 3. Viable particles of the BCG strain of *M. tuberculosis* recovered from organs of "Strong A" mice (. ., ××, ΔΔ indicate significant change from previous determination).

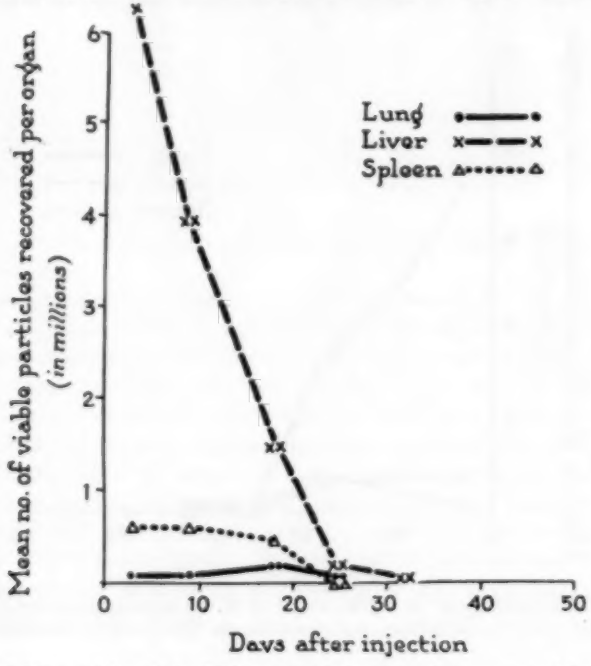

FIG. 4. Viable particles of the BCG strain of *M. tuberculosis* recovered from organs of "C57" mice (. ., ××, ΔΔ indicate significant change from previous determination).

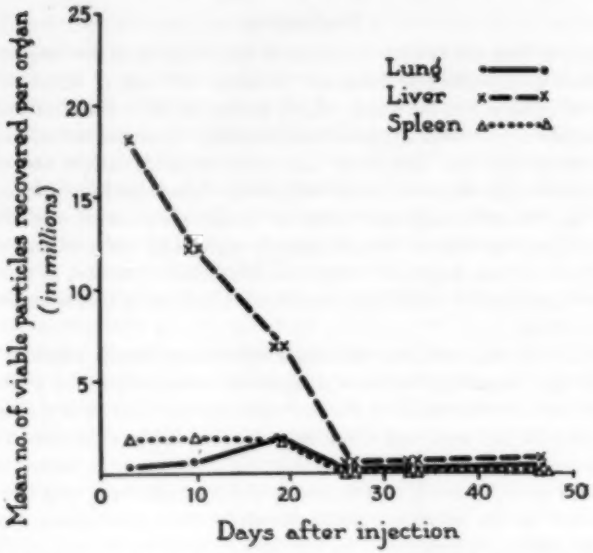

FIG. 5. Viable particles of the R1Rv strain of *M. tuberculosis* recovered from organs of "Strong A" mice (. ., ××, ΔΔ indicate significant change from previous determination).

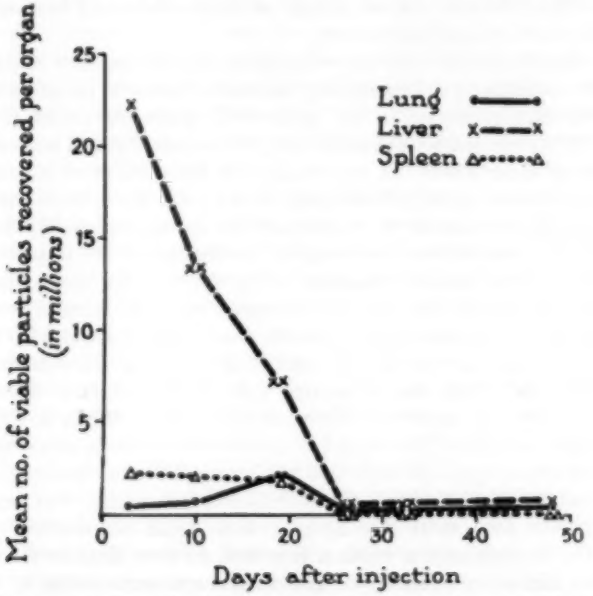

FIG. 6. Viable particles of the R1Rv strain of *M. tuberculosis* recovered from organs of "C57" mice (. ., ××, ΔΔ indicate significant change from previous determination).

Discussion

It is apparent from the results obtained in this study that the largest source of error involved in experiments designed to follow the fate of tubercle bacilli in the organs of mice is the variation which occurs in microbial content between the same organs of different animals. Furthermore, the error introduced because of this variation may be very large and may seriously affect the validity of conclusions unless the degree of such variation is determined in each experiment. This means that a sufficiently large number of animals must be sacrificed at each time interval and the organs of each homogenized and cultured individually in order to determine the degree of variation. It is difficult to see, in view of these results, how significantly valid data can be obtained using less than five mice at each time interval.

In these studies the variation obtained between replicate platings was very small and of approximately the same magnitude as that shown by Fenner for the enumeration of tubercle bacilli in Tween®-albumin cultures of mycobacteria (1). The magnitude of this source of error, however, should be determined each time the technique is employed since the possibility of its being large cannot be ignored. However, this error, if small, together with the dilution error, can be considered to be included in the variation encountered between mice, since the latter is based on the plating procedure.

Error due to clumping of bacilli when injected or due to clumps of bacilli in a homogenate at the time of plating is inherent in the technique, but this is small since Ziehl-Neelsen stained smears of suspensions and homogenates revealed single bacilli almost exclusively.

Having carefully defined the sources of error and the limits of variation, it is now possible to obtain a valid picture of the fate of tubercle bacilli in the organs of mice. The data presented in the tables and figures show that the H37Ra, BCG, and R1Rv strains of *Mycobacterium tuberculosis* suffered a similar fate in both strains of mice. From the very beginning the number of viable particles in the liver decreased rapidly. In the lung, on the other hand, there was a temporary increase in the number of viable particles for approximately two weeks, after which the number decreased rapidly. In the spleen the number of viable particles of all three strains remained fairly constant for approximately two weeks, following which the number decreased rapidly. Parallel results were obtained following administration of smaller doses of H37Ra and BCG in "Strong A" mice. The temporary rise in the number of viable particles recovered from the lungs may have been due to multiplication of the bacilli in this organ, or, on the other hand, may have been the result of a further seeding of this organ by the hematogenous route. The rapid fall in number of viable particles noted in the lung and spleen approximately two weeks after infection possibly could have been the result of an acquired immune mechanism which was beginning to function. In the liver there was an immediate rapid elimination of tubercle bacilli. In this connection it is worth noting that the 1:10 dilution of liver homogenate had an inhibitory effect on the growth of the tubercle bacilli on the culture plate. It is possible that this toxic principle was active also *in vivo* and was partly responsible for the rapid decrease in number of viable particles seen in this organ.

The finding in the present study that the H37Ra strain does not multiply in the spleens of mice is in agreement with the results of Pierce, Dubos, and Schaefer (4) and Bloch and Segal (8), but is at variance with the report of Mackaness, Smith, and Wells (5). On the other hand, the present finding that the BCG strain did not multiply in the spleens of mice is in conflict with the findings of Pierce and associates (4), Mackaness and associates (5), and Bloch and Segal (8). Moreover, the finding that the R1Rv strain did not multiply in the spleens of mice is different from the results obtained with this organism by Pierce and co-workers (4). In this connection it is of interest to note that Kanai and co-workers (9, 10) reported that a BCG strain did not multiply in either lung, liver, or spleen of intravenously infected guinea pigs, whereas the H37Ra strain did increase in numbers in the spleen of guinea pigs. It is possible that such factors as the strain of animal, the origin and state of the strain of mycobacterium employed, and the route of inoculation may in part account for these differences. However, it is believed that proper attention to the large sources of error involved in this technique also might play an important role in resolving these discrepancies.

As previously mentioned, Pierce and associates (4) divided mycobacteria into three groups, "virulent", "attenuated," and "avirulent," depending upon the ability of these microorganisms to multiply in the organs of mice.

Because of their ability to produce progressive disease, the characterization of certain mycobacteria as "virulent" is justified, but there would appear, on the basis of the present results, to be no reason for dividing mycobacteria which cannot produce progressive disease into two categories "avirulent" and "attenuated." The BCG and R1Rv strains, characterized as "attenuated" by Pierce and co-workers (4) because they had limited capacity to multiply *in vivo*, did not differ in the present study from the H37Ra strain which the same writers reported had no capacity to multiply *in vivo*. Actually, if the increase in the numbers of viable particles noted in the lungs in the present experiments is a result of actual multiplication, then all three strains possess the ability to multiply for a brief period in this organ, whereas all were unable to multiply in either the liver or spleen. Whether a microorganism should be considered "avirulent" or "attenuated" would depend upon the organ in which it happened to reside. It is important to emphasize that all three strains of tubercle bacilli behaved the same in the respective organs of two strains of mice, one of which, the "Strong A," is a mouse strain reported by Pierce and colleagues (12) to be moderately resistant to infection, the other, the "C57" strain, reported by the same investigators to be highly susceptible to tuberculous infection.

The results of the studies reported in this paper also have an important bearing on the prevailing concept that bacterial multiplication *in vivo* is essential for the production of immunity in mice to tuberculous infection when small numbers of viable mycobacteria are given (14). All three of the strains of mycobacteria employed in the present study are excellent immunizing agents and it has been shown that the smallest number of viable particles of the H37Ra strain which, under the experimental conditions, produced any significant degree of immunity was between 800,000 and 8,000,000 (15). Evidence for bacillary multiplication

in vivo is slight, limited to a moderate increase in numbers in the lungs during the first two weeks with the largest dose injected (1.0 mg.). Furthermore, the enumeration technique revealed no greater evidence of bacillary multiplication when the following smaller numbers of BCG-4 and H37Ra strains were injected intravenously—BCG-4: approximately 600,000 (0.1 mg.), 60,000 (0.01 mg)., 6,000 (0.001 mg.), and 600 (0.0001 mg.); H37Ra: approximately 4,000,000 (0.1 mg.), 400,000 (0.01 mg.), 40,000 (0.001 mg.), and 4,000 (0.0001 mg.). The above findings strongly suggest that it is the quantity of antigen injected which is of importance and that immunization will take place only when a certain minimal amount is given. There is little or no evidence to suggest that multiplication *in vivo* plays an important role in the production of a sufficient number of mycobacteria to immunize.

The possible lack of importance of bacillary multiplication to immunogenicity in mice is supported by the findings of Bloch and Segal (8) who failed to detect any adverse effect on the immunizing activity of a BCG strain of treatment of mice with isoniazid. These results were at variance with those of Dubos and coworkers (14). Bloch and Segal were at a loss to account for the discrepancy, especially since they furnished what they believed was evidence that the strain of BCG employed did multiply in the spleens of mice. Examination of the data furnished by Bloch and Segal reveals, however, no convincing evidence for actual increase in numbers of BCG organisms in the spleens of their mice. The most reasonable explanation for their findings when using isoniazid, and one which is supported by the data in the present paper, is that actually there was little or no multiplication of the BCG organisms in their mice. The isoniazid, therefore, would exert no bacteriostatic action, and the immunity produced in mice would be of the same degree as in non-isoniazid-treated vaccinated animals.

Summary

Data are presented which show the sources of error which will be encountered when attempts are made to enumerate the number of viable particles of tubercle bacilli in the organs of intravenously infected mice. The greatest source of error was found to be the variation which occurred in the microbial content between the same organs of different mice. It was found that the organs from no less than 5 mice should be cultured at each time interval, and that the organs from each mouse should be cultivated separately. The type of statistical analysis which should be made in order to obtain valid interpretation of the data also is given.

The fate of three strains of tubercle bacilli, H37Ra, R1Rv, and BCG, in the lungs, livers, and spleens of the "Strong A" and "C57" strains of mice was determined. The results revealed a similar fate for all three strains of tubercle bacilli in the respective organs of both strains of mice. There was rapid elimination of the microorganisms in the liver. There was a transient increase in the number of bacilli in the lungs for two weeks, followed by a rapid decrease. The number of bacilli found in the spleen was constant for the first two weeks, following which there was a rapid decrease in number. The same pattern was found to

occur when smaller doses of H37Ra and BCG were injected into "Strong A" mice.

The significance of these findings was discussed and it was concluded that there is no justification for the separation of these strains of tubercle bacilli into the categories "avirulent" and "attenuated" on the basis of their ability to multiply in the organs of "Strong A" or "C57" mice.

Sumario

La Enumeración de los Bacilos Tuberculosos Viables Anapatógenos de los Organos de Ratones

Los datos presentados muestran las causas de error que se encontrarán al tratar de enumerar las partículas viables de bacilos tuberculosos en los órganos de ratones infectados intravenosamente. La causa mayor de error resultó ser la variación del contenido microbiano observada entre los mismos órganos de diversos ratones. Descubrióse que debían cultivarse en el mismo período de tiempo los órganos de no menos de 5 ratones y que había que cultivar por separado los órganos de cada ratón. También se describe la clase de análisis estadístico que debe hacerse a fin de obtener una interpretación válida de los datos.

Se determinó el destino de tres cepas de bacilos tuberculosos, H37Ra, RIRv y BCG, en los pulmones, hígados y bazos de las cepas murinas "A fuerte" y "C57." Los resultados revelaron un destino semejante para las tres cepas de bacilos tuberculosos en los respectivos órganos de ambas cepas murinas. Hubo rápida eliminación de los microbios en el hígado; hubo un aumento pasajero del número de bacilos en los pulmones durante dos semanas, seguido de rápida disminución. El número de bacilos descubiertos en el bazo fué constante durante las primeras dos semanas, habiendo después de esto rápida disminución. Se observó el mismo patrón al inyectar dosis más pequeñas de H37Ra y BCG en ratones "A fuertes."

Se discute la importancia de estos hallazgos, deduciéndose que existe justificación para la separación de estas cepas de bacilos tuberculosos en categorías de "avirulentas" y "atenuadas" a base de su capacidad para multiplicarse en los órganos de ratones "A fuertes" o "C57."

Resume

La numeration des bacilles tuberculeux non pathogènes viables dans les organes de la souris

Présentation de données révélant les sources d'erreurs recontrées au cours des tentatives de numération des particules bacillaires viables dans les organes de souris infectées par voie intraveineuse. La plus grande source d'erreur est constituée par les variations du contenu microbien dans les mêmes organes de souris différentes. Il a été observé que pour chaque culture périodique il y avait lieu d'utiliser les organes provenant d'au moins 5 souris et de faire une culture séparée pour les organes de chacune des souris. Le type d'analyse statistique devant être effectué pour obtenir une interprétation valable de ces données est également décrit.

Le sort de trois souches de bacilles tuberculeux H37Ra, RIRv, et BCG dans les poumons, foies, rates des souches "Strong A" et "C57" des souris fut déterminé. Les résultats révèlent que les trois souches de bacilles tuberculeux subissaient le même sort dans les organes respectifs des deux souches de souris. L'élimination des germes du foie était rapide; à l'accroissement transitoire du nombre de bacilles dans les poumons, qui durait deux semaines, succédait leur diminution rapide. A la progression constante des bacilles découverts dans la rate durant les deux premières semaines, succédait une diminution rapide. Les mêmes modalités s'observaient quand de petites doses de H37Ra et de BCG étaient injectées dans les souris "Strong A."

La signification de ces résultats est discutée et les auteurs concluent que la séparation de ces souches de bacilles tuberculeux en catégories "avirulente" et "atténuée" sur la base de leur capacité de reproduction dans les organes des souches de souris "Strong A" et "C57" n'est pas justifiée.

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THE COURSE OF EXPERIMENTAL TUBERCULOSIS INHIBITED BY ISONIAZID[1]

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INTRODUCTION

Adequate evidence has been presented that sulfone derivatives retard experimental tuberculosis in guinea pigs (1–4), and it has been postulated that a degree of immunity was established during the course of treatment (5–7). Similarly, more recent observations have shown that isoniazid inhibits guinea pig tuberculosis much more effectively and for prolonged periods (8–10). In fact, Ferebee and Palmer (11) have considered the use of isoniazid for prophylaxis and prevention in tuberculosis, based on the observation that twenty-six weeks after challenge with tubercle bacilli guinea pigs treated with isoniazid for ten weeks had the same death rate as a normal animal group.

Further studies to test the efficiency of such treatment have confirmed these observations, but with the additional observation that guinea pigs with arrested[2] disease *eventually succumb to tuberculosis.* This report is intended not only to record the development of this process but also to portray the nature of this arrested and subsequently reactivated tuberculosis which in some aspects resembles clinical tuberculosis.

As a part of these studies, the resistance against reinfection was specifically investigated in animals with tuberculosis arrested by isoniazid.

MATERIALS AND METHODS

Two hundred and fifty-seven guinea pigs, preliminarily adjusted to the environment of the animal quarters, were separated into two weight groups ranging between 400 to 600 gm. and 600 to 800 gm. This reduced the possibility of maltreatment which occurs when there is a wide discrepancy in the size of animals in the same cage. Within each of these weight categories the animals were thoroughly randomized and placed 6 to a cage. The allocation of the cages to the five groups described below was determined separately for each weight category by lot. The location of each cage was subsequently determined by a separate lottery for the entire series for thorough randomization. Since the separate weight categories yielded no differences which were statistically significant, they receive no further consideration in this report.

The animals were divided into five groups. Three of these groups, *A*, *B*, and *C*, were injected subcutaneously in the right inguinal region with 0.1 mg. of tubercle bacilli H37Rv.

Group A consisted of 24 animals that received no treatment and served as the "early tuberculous control group."

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[1] From the Municipal Tuberculosis Sanitarium, City of Chicago, Chicago, Illinois.

[2] The criteria used to establish the clinical status of tuberculosis differ materially from those used to determine the nature and extent of the disease developed experimentally; consequently, the terminology used does not have the same significance in each instance. Specifically, in this report "arrested" is a more presumptive term as compared with the earlier clinical connotation, and implies that the process was suppressed or inhibited.

Group B consisted of 72 animals that received daily treatment for sixty days of 10 mg. of isoniazid per animal in 1 ml. of sucrose mixed with lettuce, starting seven days after the infection. This group served as the "treated tuberculous group."

Group C consisted of 71 animals and was identical with *Group B* except that fifty-two days after treatment was discontinued (or seventeen weeks after the primary infection) the surviving animals were reinfected as before with 0.1 mg. of H37Rv, and served as the "treated reinfected group."

Group D originally consisted of 60 animals that were not infected until seventeen weeks after *Groups A*, *B*, and *C* received their primary infection, and served as the "late tuberculous controls." Fifty-two of this group were alive at this time and were inoculated with tubercle bacilli simultaneously with the reinfection of *Group C*. They thus received their infection simultaneously with and at the same age as that of the survivors of *Group C* and served as late primarily infected controls for the evaluation of resistance against reinfection.

Group E. The 30 animals of *Group E* were never infected and received no drug. They served as the "healthy control group."

The nontreated groups received lettuce and sucrose solution during the period that *Groups B* and *C* received treatment.

The vast majority of animals were observed until death. Only 20, or 8 per cent of all animals, were sacrificed within the first year to establish the extent of tuberculosis during the course of the experiment. When the experiment was terminated, sixty-seven weeks after the first infection, there were living 13 animals of the treated group, 7 animals of the treated reinfected group, and 20 healthy animals. These were sacrificed. This represented 18, 10, and 60 per cent, respectively, of the original groups. The sacrificed animals and those which died spontaneously were dissected carefully to establish gross evidence of tuberculosis. In addition, the liver, lungs, and spleen were examined in each animal for microscopic evidence of tuberculosis. In a number of instances in which there was no gross evidence of tuberculosis the organs were cultured for the presence of tubercle bacilli.

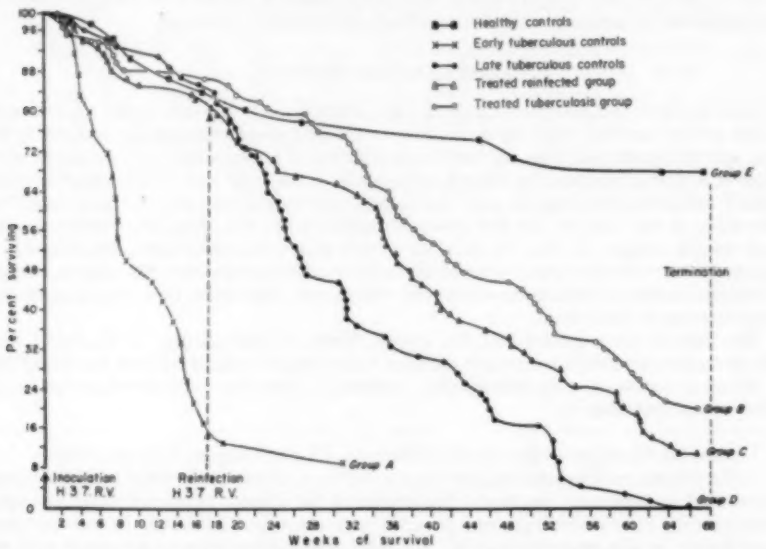

FIG. 1. Lines indicate the percentage of animals which remained alive in the various groups with time. Symbols indicate times of death.

TABLE 1

AVERAGE SURVIVAL TIME FROM THE TIME OF REINFECTION
(Except for Tuberculous Controls)

Group	Number of Animals	Primary Infection	Isoniazid Therapy	Reinfection 119 Days After Primary Infection	Average Survival Time	SE	OD	OD/SE-Diff.
					days		*days*	
A. Early tuberculous controls	24	H37Rv	None	None	90*	51	−42	2.3
B. Treated tuberculous	54	H37Rv	60 days	None	229	106	+97	4.6
C. Treated reinfected	52	H37Rv	60 days	H37Rv	207	113	+75	3.4
D. Late tuberculous controls	46	None	None	H37Rv	132	98	—	—
E. Healthy controls	30	None	None	None	378	80	+254	9.0

* Calculated from date of infection

The course of tuberculosis produced under isoniazid treatment was evaluated in three ways:

First, the number of animals surviving at various periods was determined (figure 1). This indicated, among other things, the virulence of the infecting inoculation and the protection provided by the isoniazid therapy. All of the animals included at the beginning of the study, with the exception of the 8 per cent sacrificed during the first year, are included in figure 1.

Second, the average survival times were calculated from the day of reinfection of the treated reinfected group, which occurred simultaneously with the infection of the late tuberculous controls (table 1). This provided an estimate of the resistance against reinfection when the average survival times of the different groups were statistically compared. The observed difference between the late tuberculous control group, *Group D*, and each of the other groups divided by the standard error of the difference gives the reliability quotient, OD/SE-diff. in each instance. A quotient of 2.5 or higher is acceptable since at this point the likelihood is at least 80 to 1 against these results being a chance occurrence (12). All of the animals living on the date of reinfection of *Group C* are included in table 1 with the exception of the 20 animals sacrificed during the first year of the experiment.

Third, the morphologic evidence of tuberculosis established at a specific period was used to indicate the effectiveness of isoniazid in arresting the disease (table 2). All of the animals which were inoculated with H37Rv are included in this table and the incidence of tuberculosis before and after thirty-two weeks is recorded. Thirty-two weeks was selected as the dividing line because at this point clear-cut evidence of tuberculosis appeared in *Group B*, the treated tuberculous group. In other words, in this group, tuberculosis was apparently arrested in all animals spontaneously dying or sacrificed up to this point.

RESULTS

The death rate in *Group A*, the "early tuberculous control group," was rapid and almost constant during the seventeen weeks following the inoculation with H37Rv, and seven-eighths of the animals died during this period (figure 1). In contrast, within the same period the treated animals in *Group B* showed a low mortality rate brought on mostly by intercurrent infections. These did not vary from the two other groups which had not been infected with tuberculosis at this time (*Groups D* and *E*). For practical purposes, therefore, isoniazid provided complete protection during this period.

TABLE 2

GROSS OR MICROSCOPIC EVIDENCE OF TUBERCULOSIS IN ANIMALS WHICH DIED SPONTANEOUSLY BEFORE AND AFTER 32 WEEKS FROM THE FIRST TUBERCULOUS INFECTION

Group	Number of Animals	Tuberculous at 32 Weeks				Percentage of Animals with Tuberculosis Before 32 Weeks	Tuberculous After 32 Weeks				Percentage of Animals with Tuberculosis After 32 Weeks	Percentage of All Animals with Tuberculosis
		Spontaneous Death		Sacrificed			Spontaneous Death		Sacrificed			
		Positive	Negative	Positive	Negative		Positive	Negative	Positive	Negative		
A. Early tuberculous controls	24	20	1	3	0	96	0	0	0	0	—	96
B. Treated tuberculous	72	0	17	0	5	0	21	12	10	7	62	43
C. Treated reinfected	71	3	21	0	0	12	32	5	7	3	83	59
D. Late tuberculous controls	52	27	9	4	2	74	10	0	0	0	100	80

The "late tuberculous control group" (*Group D*) also showed almost a straight-line mortality rate during the period following infection. The slope of this curve, however, was considerably flatter than that of the early tuberculous control group, presumably because the animals were seventeen weeks older when they received this inoculation.

Group C, reinfected after receiving treatment, also showed a continuous mortality rate, at a considerably lower rate, however, than the late tuberculous control group. For instance, at thirty-two weeks, 63 per cent of the former were still alive, whereas only 36 per cent of the latter group lived. In contrast, the treated but not reinfected tuberculous group (*Group B*) had a mortality rate approximately equal to the healthy controls up to twenty-nine weeks or approximately twelve weeks after the reinfection of *Group C*. At this time the animals started to die at a rather rapid rate so that eventually little difference in the number of survivors and the rate of death existed between the reinfected and nonreinfected treated groups.

The average survival time (table 1), calculated from their respective dates of infection, of the early tuberculous control group was less than that of the late tuberculous controls, the difference being, however, hardly statistically significant. In contrast, the average survival time of the isoniazid-treated group, calculated from the time of infection of the late tuberculous control group, was considerably longer. This also holds true for the reinfected group. In both instances the difference was statistically significant but was slightly less so in the reinfected group. Calculation of the observed difference between the average survival times of the reinfected and nonreinfected treated groups gave a relatively small value of twenty-two days, which proved to be statistically not significant.

During the first thirty-two weeks following the primary infection of the early tuberculous control group and the treated tuberculous *Groups B* and *C*, all of

the early tuberculous control group and four-fifths of the late tuberculous controls had died. During this period the treated tuberculous group showed no evidence of tuberculosis at autopsy (table 2). However, tubercle bacilli were obtained from the organs of 2 of the 8 animals cultured for this purpose. Meanwhile, in the treated and reinfected group, 12 per cent of the animals which had died revealed morphologic evidence of tuberculosis. Also, a number of animals in all groups had died from intercurrent infections and other causes.

Of the animals which had died or were sacrificed subsequent to the thirty-two-week period, none of the healthy controls showed tuberculosis, while 62 per cent of the treated tuberculous animals, 83 per cent of the treated reinfected group, and all of the late tuberculous group revealed morphologic evidence of tuberculosis. Ultimately, 43 per cent of all animals of the treated tuberculous group (*Group B*), 59 per cent of the treated infected group (*Group C*), 80 per cent of the late tuberculous group (*Group D*), and almost 100 per cent of the early tuberculous control group (*Group A*) had evidence of tuberculosis. Most of the late tuberculous control animals without evidence of tuberculosis died early from other causes before tuberculosis had a chance to develop.

Upon anatomic examination in the nontreated control animals severe tuberculous changes were noted and histologically the architecture of the liver appeared destroyed by extensive fibrosis and caseation. Conspicuous proliferation of the bile ducts was noted. Sometimes several contiguous lobules appeared necrotic. In the spleen coalescing tuberculous foci often involved the greater part of the parenchyma. In the lungs large areas of caseous pneumonia as well as disseminated and coalescing foci of tuberculous granulation tissue were seen. In contrast, in the treated tuberculous group, only about 20 per cent of the animals exhibiting tuberculosis after thirty-two weeks showed such severe changes. In others of the same group, either one or the other of the organs appeared spared, and when lesions were present they were represented by small tuberculous granulomas with little or no tendency to coalescence. In the treated reinfected group, 33 per cent of the animals which died thirty-two weeks after the primary infection and exhibited tuberculosis revealed a severe involvement in all organs studied histologically, while the rest showed the milder lesion just described.

Discussion

Guinea pigs infected with tubercle bacilli, proved pathogenic and fatal to control animals, failed to develop morphologically demonstrable tuberculosis in treated animals for a considerable period of time after isoniazid therapy was discontinued. The length of the period of time depended upon a number of factors which included, as shown in other studies (9–11), the dose of isoniazid given, the duration of treatment, as well as the time interval between the experimental infection and the start of the treatment. With the procedure used in this study, which entailed a time interval of seven days, no tuberculosis was demonstrable within thirty-two weeks in sacrificed animals or those which died from intercurrent infections or other causes. Since each autopsy was performed in

routine fashion, and only one block of three representative organs was taken, the absence of morphologically demonstrable tuberculosis in these experiments does not exclude the possibility of a limited tuberculous tissue change, for instance, in the nature of a primary complex. Nevertheless, approximately eight months after the infection, or six months after treatment was discontinued, the animals started to die at a more rapid rate as compared with the death rate of the healthy controls. In these animals morphologic evidence of active, either early and sometimes advanced, tuberculosis is noted, in some instances even associated with calcification or extensive fibrosis. However, in the majority of instances the tuberculosis was histologically less severe than in the tuberculous control groups. It appears thus that these animals harbor the tuberculous infection for many months without being sick, for it is significant that in a small series of animals cultures for tubercle bacilli were positive although no morphologic evidence of tuberculosis was found. Thus, guinea pig tuberculosis treated with isoniazid represents a stage of arrested rather than cured tuberculosis, at least under the experimental conditions used.

This suggests similarity with some aspects of clinical tuberculosis, even to the extent that there was a limited number of treated animals which, after sixty-seven weeks, had no evidence of tuberculosis. There was no reactivation of the primary infection and these animals apparently died of unrelated causes.

This similarity is also expressed in the response of such animals to reinfection with virulent tubercle bacilli. Reinfection is survived far longer than primary infections of animals in the same age group. Since the late tuberculous control animals, at the time of reinfection, were older than the initial tuberculous controls, they died of tuberculosis at a slower rate, presumably because of the greater resistance of the older animals, although variation in the virulence of the tubercle bacilli cannot be excluded. Nevertheless, the treated and reinfected animals, which received the same dose simultaneously with the late primary infected animals, lived significantly longer. This indicates an increased resistance to reinfection in animals with arrested guinea pig tuberculosis.

Comparison of the average survival times of the reinfected with the nonreinfected animals indicates that this protection against reinfection is only partial since the animals die at a more rapid rate when they are reinfected. Survival data, as distinguished from death rates, show the same trend, but the statistical evaluation of the former is not conclusive with the number of animals used. Moreover, the histologic changes seemed to be somewhat more extensive in the reinfected group.

At present it is not clear whether the morphologic picture is altered with reinfection as compared with that of a spontaneous activation of a previously arrested disease.

It appears that guinea pigs infected with tuberculosis and treated for a short while with isoniazid develop tuberculosis which is arrested for many months, and a partial immunity to reinfection prevails. This is somewhat analogous to the situation in human tuberculosis.

Summary

Guinea pigs infected with an effective dose of tubercle bacilli and treated for two months with isoniazid failed to show morphologic evidence of tuberculosis for approximately eight months. After this period, the majority died with evidence of tuberculosis, although it was histologically less severe in degree than in the infected control animals. This indicates that during the preceding months an arrested tuberculosis existed.

Guinea pigs in the arrested stage of tuberculosis showed a partial resistance to reinfection since they survived much longer than animals given a primary infection at the same age. The average survival time of the treated reinfected animals was shorter than that of the treated nonreinfected ones. However, this difference was not statistically significant.

Sumario

La Evolución de la Tuberculosis Experimental Inhibida por la Isoniacida

Los cobayos infectados con una dosis eficaz de bacilos tuberculosis y tratados dos meses con isoniacida no revelaron signos morfológicos de tuberculosis durante unos ocho meses. Pasado ese período de tiempo, la mayoría murió con signos de tuberculosis, aunque ésta era histológicamente menos grave que en los animales infectados como testigos. Esto indica que durante los meses precedentes existió una tuberculosis estacionada.

Los cobayos en el período estacionado de la tuberculosis mostraron resitencia parcial a la reinfección, dado que sobrevivieron mucho más tiempo que los que recibieron una infección primaria a la misma edad. El tiempo medio de sobrevivencia de los animales reinfectados tratados fué más corto que el de los no reinfectados tratados. Sin embargo, esta diferencia no tenía importancia estadística.

Resume

Le cours de la tuberculose expérimentale inhibé par l'isoniazide

Des cobayes inoculés avec une dose efficace de bacilles tuberculeux et traités par l'isoniazide pendant deux mois n'ont pas présenté les signes morphologiques de la tuberculose pendant approximativement huit mois. Au bout de cette période, la majorité a succombé et les manifestations de la tuberculose étaient présentes tout en étant moins accentuées que chez les animaux témoins inoculés. Ceci indique que durant les mois précédents il y avait en une stabilisation de la tuberculose.

Durant le stade de stabilisation de la tuberculose, les cobayes démontraient une résistance partielle à la réinfection puisqu'ils survivaient beaucoup plus longtemps que les animaux soumis à l'infection primaire au même âge. Le temps moyen de survie des animaux réinfectés et traités était plus réduit que celui des animaux non réinfectés traités. Toutefois, cette différence était sans valeur statistique.

Acknowledgment

The writers wish to thank Dr. M. J. Schiffrin of Hoffmann-La Roche, Inc., Nutley, New Jersey, for the isoniazid (Rimifon®) used in these experiments.

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A CONVENIENT METHOD FOR MEASURING VENTILATION[1,2]

Based upon the Venturi Principle

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INTRODUCTION

Tests of ventilation yield much information of value for the management of patients with pulmonary and cardiac disease. It is fortunate that most of these tests are not difficult to perform and therefore lend themselves readily to clinical use by internists and surgeons.

Maximal breathing capacity has become accepted as the most useful and important of the dynamic tests of ventilation (1). It measures the integrated ability of the entire respiratory apparatus to perform maximal ventilation *per unit time*, and this capacity of the patient for maximal ventilation depends upon many factors: compliance of the lungs and thorax, airway and tissue resistance, and available muscular force (2). Thus, the maximal breathing capacity test is suitable for detection of *restrictive* and *obstructive* ventilatory deficiencies, and of muscular *fatigue*. In contrast, obstruction to the flow of air and fatigue are not measured by the vital capacity, which depends upon a single maximal expiration *without regard to time*. Serial tests of maximal breathing capacity may demonstrate deterioration of ventilatory ability in relation to progressing disease or may indicate functional improvement as the result of treatment.

In some patients it may be advisable to measure ventilation while performing exercise in order to calculate breathing reserve or to determine if a patient is hyperventilating in relation to his need for oxygen. The walking ventilation test, carried out in a manner to imitate the act of walking slowly on the level, is particularly suitable for patients (3).

Determination of the maximal breathing capacity or walking ventilation requires similar equipment (3, 4). The awkward valves and the cumbersome meters, bags, and spirometers necessary for the performance of these tests have limited their clinical use. An apparatus combining features of accuracy, low resistance to breathing, compactness and portability, convenience of use, and ease of cleaning would encourage more widespread utilization of these simple, helpful tests. These qualities are found, in large part, in the instrument to be described.

TECHNIQUE

The Venturi tube is a cylinder with an internal diameter which becomes progressively smaller from each end to the throat at the middle of the tube.

[1] From the Laurel Heights State Tuberculosis Sanatorium, Shelton, Connecticut.

[2] Presented at the annual meeting of the Eastern Section of the American Trudeau Society, Schenectady, New York, November 10, 1956.

Velocity of gas or fluid flowing through the tube is greater at the narrow throat, in accordance with Bernoulli's theorem:

$$Q = AV, \text{ where:}$$

Q = quantity per unit of time;

A = area of cross-section of the stream; and

V = average velocity.

A drop in the pressure head observed at taps or side arms placed near the entrance and at the throat reflects differences in velocity in the tube at these points. Applying this principle, engineers and physicists for many years have utilized the Venturi tube (or meter) to measure the flow of gases or fluids (5).

In 1951, Maloney, Silverman, and Whittenberger described the use of a Venturi meter to determine minute ventilation of patients in a respirator or while undergoing anesthesia (6). A differential draft gauge connected to the side arms was calibrated to indicate ventilation through the tube in liters per minute.

Cooper, Blakemore, and Johnson substituted a less expensive fluid manometer for the draft gauge and recommended the use of the Venturi meter for the clinical measurement of maximal breathing capacity (7). As the patient breathed through the tube a reading on the manometer was noted and this was converted to maximal breathing capacity by reference to a calibration curve. In actual use it was found that the fluid in the manometer fluctuated slightly with rapid inspiration and expiration even when dampening devices were inserted into the system. Also, by supreme effort the patient could attain a peak manometer reading at the beginning of a test period but could not maintain a steady level for the remainder of the fifteen seconds. These two variations made correct reading of the manometer difficult.

At any instant a reading of the manometer of the Cooper-Blakemore-Johnson instrument indicates the mean air-flow rate of only the preceding few breaths. On the other hand, the maximal breathing capacity is defined as the maximal volume of gas that can be breathed *per unit time*.

The Ventube

In practically every laboratory a modified Venturi tube with a single side arm at the throat is attached to a water faucet. When water flows through the tube, suction is created through the side arm and this plain device serves as a very effective suction pump or aspirator.

Flow of air through a similar tube will aspirate air through the side arm. It seemed evident that, if the *volume* of air aspirated through the side arm could be measured and related accurately to the volume of air passing through the tube, this would provide a convenient method for determining ventilation. Tubes with throats and side arms of various internal diameters were tried and one was selected which offered low resistance to ventilation, combined with suitable volume of aspiration of air for standard test periods.[3]

[3] The modified Venturi tubes were made by the J. H. Emerson Company, 22 Cottage Park Avenue, Cambridge 40, Massachusetts.

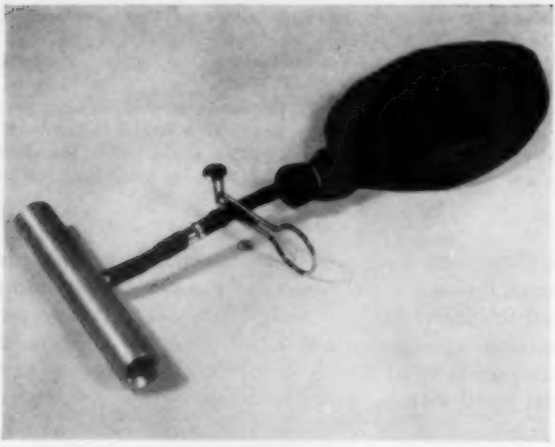

FIG. 1. The ventube. For the purpose of filling or emptying the balloon it can be detached from the modified Venturi tube at the Luer-lok® connection.

Exact measurement of volume of air aspirated through the side arm presented a problem. A small, sufficiently sensitive meter was considered but was rejected as being too expensive and fragile, and a spirometer connected to the side arm would have impaired the compactness and portability of the instrument. Measuring the volume of air in a detachable balloon before and after each test period provided a simple solution (figure 1). The modified Venturi tube used in this manner was given the short and convenient name "ventube."

In order to ascertain the value of the instrument for clinical use it was necessary: (*1*) to calibrate the ventube for maximal breathing capacity and walking ventilation over a wide range of rates and volumes; (*2*) to measure resistance to air flow through the throat of the tube; and (*3*) to test the maximal breathing capacity of patients with the ventube and compare the results with those obtained in the same patients by other methods.

Calibration of the instrument required simultaneous measurement of the volume of air aspirated through the side arm and the volume of air flowing back and forth through the tube. Experimentation showed that it was not possible to calibrate by breathing through the ventube placed in tandem with a one-way valve and bag or with a spirometer since these connections altered the tube and side arm pressure relationship. Instead, it was necessary to use a reciprocating pump to move known quantities of air through the tube and then to measure the volume of air removed from the balloon at the conclusion of each test period.

Apparatus

Specifications of the modified Venturi tube are as follows:

Length of tube: six inches
Internal diameter at ends: $^{35}/_{64}$ of an inch
Length of throat: $1^{3}/_{4}$ inches
Internal diameter at throat: $^{26}/_{64}$ of an inch

Internal diameter of side arm: 0.070 inch, with a 0.042 inch constriction at the junction of the side arm and the throat of the tube.

The balloon[4] was made of thin rubber and had a flaccid capacity of approximately 1,000 cc.

The pump was a reciprocating, piston-type, hand-operated, airtight pump, with a diameter of five inches, and was mounted vertically on a rigid stand. Stroke volume could be set from 114 cc. to 2,653 cc., and the dead space at the end of the stroke was 170 cc. Stroke volume was measured under static conditions by connecting the pump to a spirometer.

Observations

Calibration of the ventube: To approximate conditions existing when a patient breathes through the ventube, the tube was inserted directly into the outlet of the pump, and the pump was operated in a manner to imitate performance of maximal breathing capacity or walking ventilation as closely as possible. One cycle of the pump was equivalent to a breath (inspiration and expiration) and the cycles were timed with an electric metronome. Thus,

$$\text{Ventilation (liters per minute)} = \frac{\text{cycles per minute} \times \text{stroke volume (cc.)}}{1{,}000}.$$

Calibration for maximal breathing capacity was performed in the range from 20 to 209 liters per minute, at rates of 20, 40, 60, 80, 100, and 120 cycles per minute and using stroke volumes from 228 cc. to 2,653 cc. Each calibration test for maximal breathing capacity was done in duplicate.

Calibration for walking ventilation was performed in the range from 10 to 39 liters per minute, at rates of 15, 20, and 30 cycles per minute and using stroke volumes from 694 cc. to 1,275 cc. Each calibration test for walking ventilation was done in triplicate.

At the start of each test a known quantity of air was placed in the balloon. To do this the balloon with its rubber tube was connected to a 100 cc. syringe and stopcock. The balloon was first emptied by alternately withdrawing air into the syringe and expelling it through the three-way stopcock. When less than 100 cc. of air remained in the balloon the syringe was elevated into the vertical position and the barrel of the syringe was allowed to fall by gravity until all air was exhausted from the balloon (figure 2). This gave a reproducible end state of emptiness. The syringe was lubricated lightly with sewing machine oil to make it airtight and to ensure ease of motion of the barrel. By using the syringe and stopcock, a carefully measured volume of air was then injected into the balloon using multiples of 100 cc. and a minimum of 300 cc. After this, the balloon was sealed off with a pinch clamp on the rubber tube and the balloon and tube were connected to the side arm of the Venturi tube (figure 1).

For an actual test the operation of the pump was begun first. When the pump was synchronized with the metronome, the pinch clamp was opened, air was aspirated from the balloon for an exact number of strokes, and the clamp was then promptly closed. The test period for maximal breathing capacity was fifteen seconds and for walking ventilation was one minute.

At the conclusion of the test the balloon and its tube were removed from the

[4] Darex J-10 balloon, manufactured by Dewey and Almy Chemical Company, Cambridge 40, Massachusetts.

FIG. 2. Emptying the balloon. The last few cubic centimeters of air are removed from the balloon by elevating the 100 cc. syringe into the vertical position and allowing the barrel to descend by gravity. This gives a reproducible end state of emptiness.

side arm of the ventube and attached to the syringe and stopcock. Volume of air remaining in the balloon was measured, care being taken to obtain a final reading at the "end state" as described above. The cubic centimeters of air removed from the balloon during the test period were obtained by subtraction.

The following is an example of a calibration test for maximal breathing capacity:
Stroke volume of pump: 933 cc.
Metronome setting and pump rate: 100 cycles per minute
Simulated maximal breathing capacity: 93.3 liters per minute (933 cc. × 100)
Test period: fifteen seconds
Pump cycles in fifteen seconds: 25 ($^1/_4$ × 100)
Volume of air in balloon before test: 500 cc.
Volume of air in balloon after test: 160 cc.
Volume of air removed during test: 340 cc.

Example of calibration test for walking ventilation:
Stroke volume of pump: 1,036 cc.
Metronome setting and pump rate: 20 cycles per minute
Simulated walking ventilation: 20.7 liters per minute (1,036 cc. × 20)
Test period: one minute
Pump cycles in test period: 20
Volume of air in balloon before test: 400 cc.
Volume of air in balloon after test: 159 cc.
Volume of air removed during test: 241 cc.

TABLE 1

Calibration of Ventube

Simulated Maximal Breathing Capacity

Stroke Volume	Rate 20 cycles/min.		Rate 40 cycles/min.		Rate 60 cycles/min.		Rate 80 cycles/min.		Rate 100 cycles/min.		Rate 120 cycles/min.	
	Maximal Breathing Capacity	Cc. Removed	Maximal Breathing Capacity	Cc. Removed	Maximal Breathing Capacity	Cc. Removed	Maximal Breathing Capacity	Cc. Removed	Maximal Breathing Capacity	Cc. Removed	Maximal Breathing Capacity	Cc. Removed
cc.	*L.*		*L.*		*L.*		*L.*		*L.*		*L.*	
228									22.8	72	27.4	91
										73		93
342							27.4	88	34.2	116	41.0	143
								88		116		142
							27.4	91	34.2	115	41.0	142
								91		116		144
456					27.4	85	36.5	124	45.6	162	54.7	196
						85		123		161		197
570			22.8	71	34.2	112	45.6	160	57.0	207	68.4	250
				70		111		161		204		250
694			27.8	86	41.6	140	55.5	200	69.4	251	83.3	308
				88		140		205		254		305
798			31.9	103	47.9	166	63.8	236	79.8	295	96.0	354
				102		167		235		297		353
933			37.3	121	56.9	196	74.6	275	93.3	340	112.0	408
				120		196		276		340		405
1,036	20.7	59	41.4	140	62.2	222	82.9	313	103.6	380	124.3	456
		59		141		223		308		380		456
1,161	23.2	68	46.4	163	69.7	250	92.9	342	116.1	425	139.3	505
		69		161		250		340		424		507
1,275	25.5	74	51.0	178	76.5	282	102.0	378	127.5	473	153.0	558
				178		280		379		472		558
1,389	27.8	83	55.6	197	83.3	300	111.1	411	138.9	505	166.7	610
		84		197		300		408		503		604

1,513	30.3	94	60.5	212	90.8	328	121.0	434	151.3	542	181.6	647
		92		212		326		435		545		648
1,617	32.3	101	64.7	232	97.0	353	129.4	472	161.7	584	194.0	690
		102		232		350		473		586		694
1,741	34.8	114	69.6	250	104.5	377	139.3	506	174.1	630	208.9	757
		112		249		378		510		629		753
1,845	36.9	120	73.8	271	110.7	401	147.6	530	184.5	669		
		119		270		398		529		669		
1,949	39.0	130	78.0	284	116.9	414	155.9	560	194.9	697		
		128		284		417		560		699		
2,073	41.5	138	82.9	301	124.4	444	165.8	597	207.3	745		
		138		302		447		594		744		
2,197	43.9	146	87.9	321	131.8	470	175.8	628				
		147		320		473		627				
2,291	45.8	156	91.6	339	137.5	489	183.3	660				
		154		338		494		663				
2,425	48.5	165	97.5	346	145.5	521	194.0	702				
		167		346		522		698				
2,539	50.8	177	101.6	371	152.3	544	203.1	728				
		174		372		546		728				
2,653	53.1	181	106.1	393	159.2	580	212.2	771				
		182		393		582		770				
Slope	0.2578		0.2589		0.2717		0.2766		0.2745		0.2760	
S.E. of slope	0.00181		0.00154		0.00134		0.00140		0.00117		0.00140	
Regression equation	Y = 5.92 + 0.2578 (X)		Y = 4.99 + 0.2589 (X)		Y = 2.77 + 0.2717 (X)		Y = 0.05 + 0.2766 (X)		Y = 1.03 + 0.2745 (X)		Y = 0.30 + 0.2760 (X)	

X = cc. air removed from balloon.
Y = maximal breathing capacity—liters per minute.

Calibration data for maximal breathing capacity are shown in table 1. Very good agreements were obtained in duplicate tests with variations seldom exceeding 1 per cent. For each rate the plotted values form practically a straight line. Slopes for the higher rates of 60, 80, 100, and 120 per minute paralleled each other closely (0.272, 0.277, 0.275, and 0.276). Slopes for the lower rates of 20 and 40 per minute also paralleled each other but were slightly steeper (0.258, 0.259) than those for the higher rates. The standard error of the slope was small for each rate.

Calibration data for walking ventilation are contained in table 2. Although good agreement was obtained in triplicate tests, the variation, usually less than

TABLE 2

CALIBRATION OF VENTUBE
Simulated Walking Ventilation

Stroke Volume	Rate 15 cycles/min.		Rate 20 cycles/min.		Rate 30 cycles/min.	
	Walking Ventilation	Cc. Removed	Walking Ventilation	Cc. Removed	Walking Ventilation	Cc. Removed
cc.	L.		L.		L.	
694	10.4	76	13.9	134	20.8	241
		80		136		245
		85		135		243
694	10.4	83			20.8	239
		86				250
		86				244
798	12.0	105	16.0	168	23.9	288
		108		165		287
		106		160		287
933	14.0	131	18.7	207	28.0	341
		134		206		340
		138		206		342
933	14.0	126				
		131				
		131				
1,036	15.5	156	20.7	241	31.1	404
		157		241		407
		158		241		406
1,161	17.4	180	23.2	270	34.8	448
		182		270		446
		183		272		450
1,275	19.1	200	25.5	313	38.3	518
		213		318		524
		212		316		520
Slope	0.068		0.065		0.064	
S.E. of slope	0.00109		0.00096		0.00106	
Regression equation	Y = 4.84 + 0.068 (X)		Y = 5.29 + 0.065 (X)		Y = 5.47 + 0.064 (X)	

X = cc. air removed from balloon.
Y = walking ventilation—liters per minute.

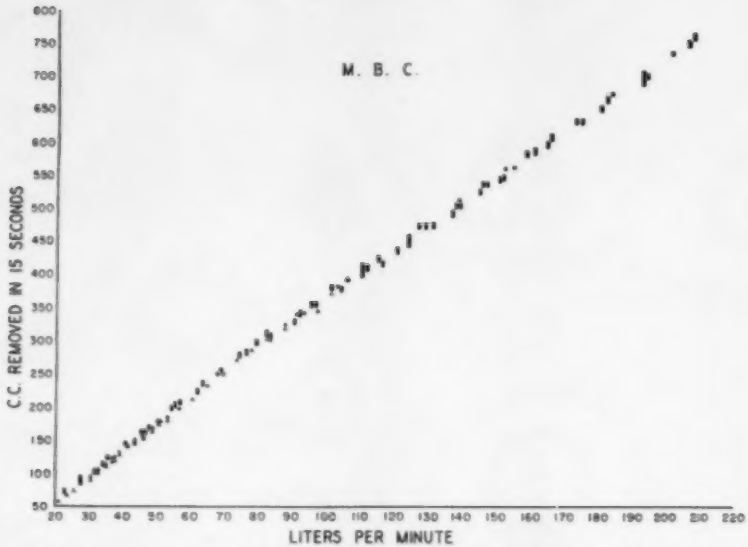

FIG. 3. Calibration of the ventube for maximal breathing capacity. The cubic centimeters of air removed from the balloon in the fifteen-second test period are plotted against simulated maximal breathing capacity (liters per minute) through the tube. Values for faster rates of 60, 80, 100, and 120 per minute are indicated by squares. Slower rates of 20 and 40 per minute are shown by triangles.

3 per cent, was slightly greater than for maximal breathing capacity. The plotted values for each rate form practically a straight line and the slopes for the three rates of 15, 20, and 30 per minute were closely parallel (0.0683, 0.0647, and 0.0640). The standard error of the slope was small for each rate.

When calibration data for maximal breathing capacity for *all* respiratory rates were plotted together the composite pattern assumed a very slight curve (figure 3). This was due to the minor difference in slope for the higher rates of 60, 80, 100, and 120 per minute as compared with the slower rates of 20 and 40 per minute. Conversion tables were made from the observed values on this graph (table 3). With separate tables for fast rates (60 to 120 per minute) and for slow rates (20 and 40 per minute) deviations of observed values from the values listed in the conversion tables were minor. Deviation greater than 2 per cent occurred only seventeen times in 217 tests and never exceeded 5 per cent.

In this laboratory 85 per cent of 337 tests of maximal breathing capacity were performed by patients using rates ranging from 60 to 120 per minute. Seven per cent of the tests were carried out at rates lower than 60, and 8 per cent at rates higher than 120. Therefore, the conversion table for the higher rates can be used for the great majority of patients.

A composite graph for walking ventilation at *all* rates of respiration tested is shown in figure 4. A conversion table for walking ventilation was made from the

TABLE 3

CONVERSION TABLES FOR MAXIMAL BREATHING CAPACITY

Cc. Removed	0	100	200	300	400	500	600	700
Rates of More than 60 per Minute								
0		30.0	56.0	81.5	110.0	138.5	166.5	194.5
5		31.5	57.0	83.0	111.5	140.0	168.0	196.0
10		33.0	58.5	84.0	113.0	141.5	169.5	197.5
15		34.0	60.0	85.5	114.0	143.0	171.0	199.0
20		35.5	61.0	87.0	115.5	144.5	172.0	200.0
25		36.5	62.0	88.5	117.0	145.5	173.5	201.5
30		38.0	63.5	90.0	118.5	147.0	175.0	203.0
35		39.0	65.0	91.5	120.0	148.5	176.0	204.5
40		40.5	66.0	93.0	121.5	150.0	177.5	206.0
45		41.5	67.5	94.0	123.0	151.5	179.0	207.5
50	Maximal Breathing Capacity–Liters	43.0	68.5	95.5	124.5	153.0	180.5	208.5
55		44.5	70.0	97.0	126.0	154.0	182.0	210.0*
60	20.0*	45.5	71.5	98.5	127.5	155.5	183.0	
65	21.5	47.0	72.5	100.0	128.5	157.0	184.5	
70	22.5	48.0	74.0	101.0	130.0	158.0	186.0	
75	24.0	49.5	75.0	102.5	131.5	159.5	187.5	
80	25.0	50.5	76.5	104.0	133.0	161.0	189.0	
85	26.5	52.0	77.5	105.5	134.5	162.5	190.5	
90	27.5	53.0	79.0	107.0	136.0	164.0	192.0	
95	29.0	54.5	80.0	108.5	137.0	165.0	193.5	
Rates of Less than 60 per Minute								
0		31.5	57.0	83.0	108.5			
5		32.5	58.5	84.5	110.0			
10		34.0	60.0	85.5	111.0			
15		35.0	61.0	87.0				
20		36.5	62.5	88.5				
25		38.0	63.5	89.5				
30		39.0	65.0	90.5				
35		40.5	66.5	92.0				
40		41.5	67.5	93.0				
45	Maximal Breathing Capacity–Liters	43.0	69.0	94.5				
50		44.0	70.0	95.5				
55	20.0*	45.5	71.5	97.0				
60	21.0	47.0	72.5	98.0				
65	22.5	48.5	74.0	99.5				
70	23.5	49.5	75.0	101.0				
75	25.0	51.0	76.5	102.0				
80	26.0	52.0	78.0	103.5				
85	27.5	53.0	79.0	105.0				
90	28.5	54.5	80.5	106.0				
95	30.0	56.0	82.0	107.5				

* Interpolated.

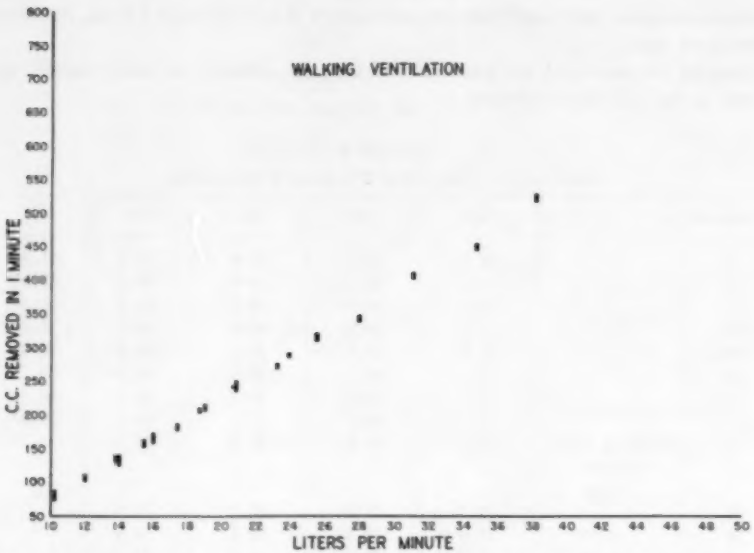

FIG. 4. Calibration of the ventube for exercise (walking) ventilation. The cubic centimeters of air removed from the balloon in the one-minute test period are plotted against simulated walking ventilation (liters per minute) through the tube, at rates of 15, 20, and 30 per minute.

observed values of this curve (table 4). Deviations of observed values from values listed in the conversion table were minor. Deviation greater than 2 per cent occurred only fifteen times in 63 tests and never exceeded 6 per cent.

Resistance to flow of air through the ventube: Resistance to flow of air through the ventube was measured by Dr. Inga Lindgren, using the method described by Gaensler, Maloney, and Björk (8). In table 5 flow resistance for the ventube is compared with values obtained for three commonly used low-resistance valves: the Hans Rudolph high velocity valve, the double "J" valve, and the large double Douglas valve. Flow resistance through the ventube is two to three times greater than that encountered in the best low-resistance valve, the large double Douglas valve. It offers twice as much resistance as the double "J" valve and somewhat less than twice as much resistance as the Hans Rudolph valve.

Lindgren, Cugell, and Gaensler (9) point out the fact that tubing used in conjunction with breathing apparatus may offer more resistance than the valves themselves. Tube resistance varies directly with the length of the tube and in inverse ratio to the fifth power of the diameter of the tube. Data on resistance of various tubes, 76 cm. long and held in a straight line, are included in table 5. Resistance to air-flow through the ventube was probably less than resistance encountered with combinations of low-resistance valves *plus* commonly used corrugated or smooth 1.9 cm. internal diameter tubing. Air-flow resistance in the

ventube exceeded valve and tube resistance only when the large 3.0 cm. diameter tubes were used.

Opening pressure and the possibility of leakage, inherent in many valves, are absent in the valveless ventube.

TABLE 4

CONVERSION TABLE FOR WALKING VENTILATION

Cc. Removed	0	100	200	300	400	500
0		11.7	18.4	24.9	31.2	37.6
5		12.0	18.7	25.2	31.5	37.9
10		12.4	19.0	25.5	31.8	38.2
15		12.7	19.4	25.8	32.1	38.6*
20		13.0	19.7	26.1	32.4	38.9*
25		13.3	20.0	26.4	32.8	39.2*
30		13.7	20.4	26.8	33.1	39.5*
35		14.0	20.7	27.1	33.4	39.8*
40	Walking Ventilation–Liters	14.4	21.0	27.4	33.7	40.1*
45		14.7	21.4	27.9	34.0	
50	8.4*	15.0	21.7	28.1	34.4	
55	8.7*	15.4	22.0	28.4	34.7	
60	9.0*	15.7	22.4	28.7	35.0	
65	9.4*	16.0	22.7	29.0	35.3	
70	9.7*	16.4	23.0	29.3	35.7	
75	10.0*	16.7	23.4	29.6	36.0	
80	10.4	17.0	23.7	30.0	36.3	
85	10.7	17.3	24.0	30.3	36.6	
90	11.0	17.7	24.3	30.6	36.9	
95	11.3	18.0	24.6	30.9	37.2	

* Interpolated.

TABLE 5

AIR-FLOW RESISTANCE IN VENTUBE, VALVES, AND TUBING
(Resistance Expressed in Cm. of Water)

	Rate of Air Flow—Liters per Minute					
	100 L.	200 L.	300 L.	400 L.	500 L.	600 L.
	cm.	cm.	cm.	cm.	cm.	cm.
Ventube	2.0	5.2	10.7	19.2	34.0	—
Hans Rudolph valve	1.4	4.3	7.4	11.6	16.5	22.0
Double "J" valve	0.7	2.5	5.3	10.3	16.9	23.4
Large double Douglas valve	0.9	2.8	4.8	8.0	10.6	13.5
Standard corrugated tube—1.9 cm.	1.5	4.5	10.5	24.0	39.0	—
Smooth rubber tube—1.9 cm.	1.0	2.5	5.0	9.5	14.5	22.0
Wide corrugated tube—3.0 cm.	0.5	1.5	3.0	5.5	9.0	13.5
British cloth-covered corrugated tube—3.0 cm.	0	0.5	1.5	2.5	4.0	7.0

Data from Lindgren, I., and Lindgren, I., Cugell, D. W., and Gaensler, E. A.

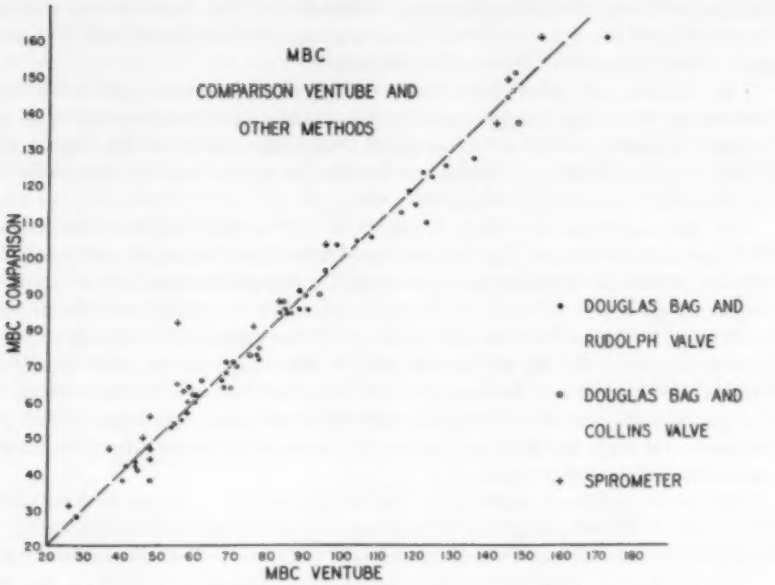

FIG. 5. Maximal breathing capacity by the ventube compared with other methods.

Actual tests of maximal breathing capacity obtained by the ventube as compared with other methods: Patients with varying degrees of impairment of ventilation performed maximal breathing capacity first with the ventube and then by one of three other commonly used methods: Hans Rudolph high velocity valve, with Douglas bag (34 patients); Collins high velocity "J" valve, with Douglas bag (16 patients); and nine-liter Collins Respirometer (20 patients). A single 1.9 cm. corrugated tube connected the valves to the Douglas bag. Two such tubes led to the respirometer. Duplicate tests were performed and the highest values obtained for each method were compared. Test periods were for fifteen seconds (except for twelve seconds with the respirometer), and suitable rest was taken between tests.

Results are shown in figure 5. Good agreement was found between values obtained by the ventube and by each of the three methods with which it was compared.

DISCUSSION

Calibration data and comparison of performance establish the ventube as an instrument sufficiently accurate for clinical use in determining maximal breathing capacity and exercise ventilation of patients. New tubes must be machined to close tolerance and their calibration should be checked before placing them in use. There are no parts to assemble or to get out of order, and accuracy is maintained as long as the tube and its side arm are kept clean. In the period of eight

months following the original testing, calibration of the ventube was checked repeatedly and was found to be the same although the instrument had been used and cleaned numerous times in the interval.

The main advantage of the ventube is its compactness and portability. The instrument in its case is approximately the size of a sphygmomanometer. It can be used for determination of the maximal breathing capacity at the bedside and in the physician's office, or to measure ventilation at any level of exercise (walking, step-test, treadmill, climbing, et cetera).

The instrument is convenient to use, both for the physician and the patient, and this fact encourages duplicate tests and subsequent follow-up examinations. To test ventilation requires only three rapidly performed steps: (*1*) a measured volume of air is injected into the balloon with a 100 cc. syringe and the balloon is attached to the side arm of the ventube, (*2*) the patient breathes through the ventube (figure 6) for the test period, and (*3*) the volume of air remaining in the balloon is measured and the amount of air removed from the balloon during the test period is converted to ventilation by referring to the conversion tables. Adjustments for body temperature, barometric pressure, or water saturation are not necessary for clinical purposes.

For determination of walking ventilation, the patient walks on the level at the slow pace of 180 feet per minute. The procedure, as originally described, took four minutes—a one-minute "warm-up period," followed by a three-minute test

FIG. 6. Using the ventube. The patient can breathe through either end of the tube. The pinch-clamp is opened and closed at the beginning and conclusion of the test period.

period during which the patient breathed through a one-way valve into a Douglas bag (3). The air collected in the bag was measured and the result, expressed in liters per minute, was the average ventilation for the three minutes of the test period. When using the ventube for measuring walking ventilation, it is more convenient to increase the length of the "warm-up period" to two minutes and to reduce the succeeding test period to one minute. For determining ventilation during more strenuous exercise in which the minute ventilation exceeds 40 liters, the test period can be decreased to fifteen seconds and the conversion table for maximal breathing capacity can be used.

The simplicity of the instrument is psychologically reassuring to patients who tend to be made apprehensive by the sound and movement of more complicated laboratory equipment.

Cleaning the ventube is accomplished easily by placing the metal tube in alcohol. After soaking, excess alcohol is shaken from the tube and any moisture remaining in the small side arm is blown out with a rubber bulb. Occasionally, it may be necessary to cleanse the inside of the tube with a small, soft, test-tube brush.

Summary

The volume of air aspirated through the side arm of a modified Venturi tube can be related accurately to the volume of air passing to and fro through the throat of the tube. Calibration data and comparison with other methods show that this simple device is sufficiently accurate for the clinical measurement of maximal breathing capacity and exercise ventilation.

The instrument (a ventube) has additional advantages of being compact and portable, convenient to use, and easy to clean. Resistance to breathing through the tube is comparable with that encountered in low resistance valves together with their connecting tubing.

Sumario

Método Conveniente para Medir la Ventilación, Basado en el Principio de Venturi

El volumen de aire aspirado a través del brazo lateral de un tubo de Venturi modificado puede relacionarse exactamente con el volumen de aire que entra y sale por la garganta del tubo. Los datos de calibración y la comparación con otros métodos revelan que este sencillo aparato es de suficiente exactitud para la medición clínica de la capacidad respiratoria máxima y la ventilación al ejercicio.

El instrumento (ventubo) posee además las ventajas de ser compacto y portátil, cómodo para empleo y fácil de limpiar. La resistencia a la respiración a través del tubo es comparable a la encontrada en las válvulas de poca resistencia unidas al tubo conector.

Resume

Une méthode commode de détermination de la ventilation, fondée sur le Principe de Venturi

Il est possible d'établir une relation adéquate entre le volume de l'air aspiré dans la branche latérale d'un tube de Venturi modifié et le volume de l'air entrant et sortant par l'ouverture du tube. Les données de l'étalonnement et la comparaison avec d'autres méthodes montrent que ce simple dispositif est suffisamment précis pour déterminer cliniquement la capacité respiratoire maxima et la ventilation à l'effort.

L'appareil (tube de Venturi) possède en outre l'avantage d'être peu emcombrant, facile à transporter, à utiliser et à nettoyer. La résistance à la respiration dans le tube est comparable à celle rencontrée dans les soupapes à faible résistance avec tubes raccordés.

Acknowledgment

The writers wish to express their gratitude to Dr. Inga Lindgren, Thorndike Memorial Laboratory, Boston City Hospital, for studying the flow resistance of the ventube and for permission to use data on flow resistance in valves and tubing. Dr. Lindgren also independently confirmed the calibration of the ventube for maximal breathing capacity.

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Case Reports

PULMONARY HISTIOCYTOSIS X[1]

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INTRODUCTION

Diffuse disease of the lungs has numerous etiologies and the list continues to grow. In recent years histiocytosis X has been added. This entity has been precipitated from the supersaturated group of granulomas by Farber, Mallory, and Lichtenstein since 1940 (1). The term was invented by Lichtenstein to denote a peculiar inflammatory histiocytosis of unknown cause manifested by several clinical syndromes previously known as Letterer-Siwe disease, Hand-Schüller-Christian disease, and eosinophilic granuloma.

Cumulative experience has shown that these clinical pictures are all expressions of the same disease, differing only as to course and localization of lesions. There is a spectrum of cases from the fulminating, usually fatal, acute reticuloendotheliosis of Letterer-Siwe seen most often in infants, through the chronic disseminated disease of Hand-Schüller-Christian, to the benign localized eosinophilic granuloma of young adults. This integration of concept has been aided to a great extent by biopsy, especially of the pulmonary lesions, in a small number of cases since 1951 (1–8). Synthesis of biopsy and autopsy material is also helping to clarify the pathogenesis of the rare so-called "honeycomb lung" (9–11).

The following case is presented as an example of pulmonary histiocytosis X with "honeycomb lung" in the early stage of disease, as demonstrated by bilateral lung biopsy.

CASE REPORT

In October, 1954, a 22-year-old white male was admitted for study to the pulmonary disease section of an Air Force hospital with the presumptive diagnosis of tuberculosis. The patient had grown up in Montana with no known exposure to tuberculosis. Family history and past medical history were unrevealing. In January, 1950, he had first enlisted in the United States Air Force and had served in the Zone of the Interior until separation in September, 1953. Several months prior to separation he had begun to lose some weight and in January, 1954, he began to have transient attacks of chest pain, pleural in type, sometimes on one side and sometimes on the other side. They occurred at irregular intervals, approximately once a month, and lasted several days but were not associated with dyspnea.

The patient re-enlisted in June, 1954, and developed a cough for several weeks. This was followed by an attack of pain in the left chest and a roentgenogram showed a moderate left spontaneous pneumothorax (figure 1). He was hospitalized at an Air Force base in Washington for a month and treated with bed rest. In September, 1954, he was again hospitalized because of bilateral pneumothorax and, because infiltrations were noted in both lungs, he was transferred to a pulmonary disease center for study.

Systemic review revealed that he had had several attacks of epigastric pain suggestive of peptic ulcer during the previous two years.

Physical examination showed a well-developed, slender young male with no detectable abnormalities.

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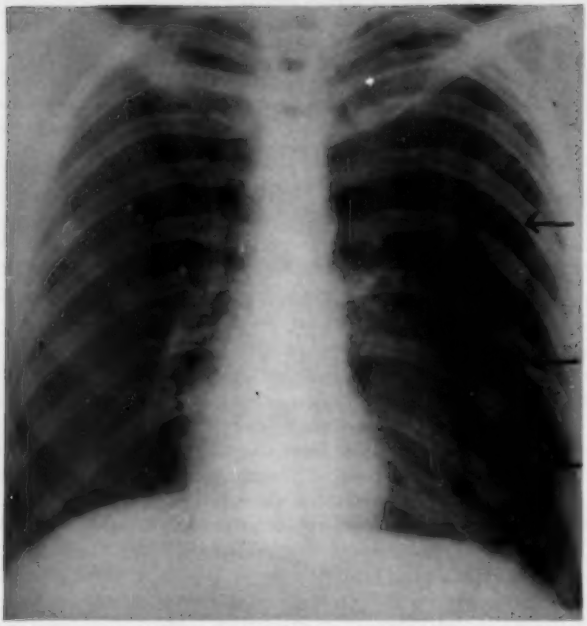

FIG. 1. Postero-anterior chest roentgenogram showing diffuse nodulation with left pneumothorax.

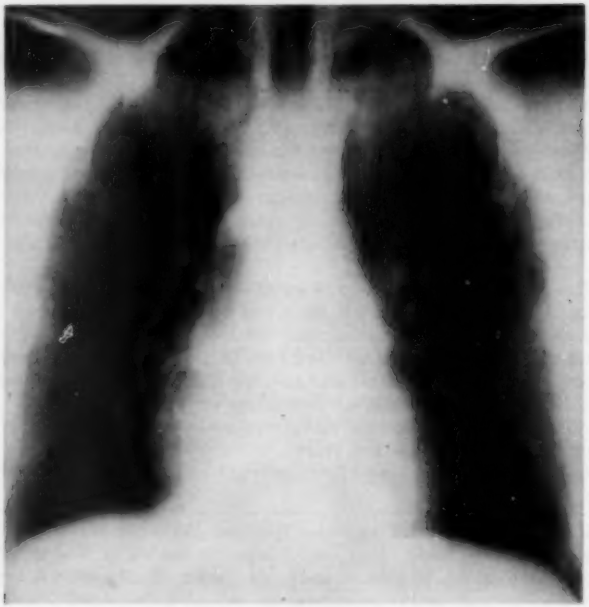

FIG. 2. Planigram of the chest showing cystic lesions in the left upper lung field.

A roentgenogram of the chest (figure 1) showed scattered small patchy densities throughout both lungs; they were somewhat more prominent in the upper lung fields, with a suggestion of honeycombing, particularly on the left, as demonstrated by planigram (figure 2). The initial leukocyte count was 15,000 with 90 per cent neutrophils, but a repeat examination was normal, as was the determination of hemoglobin. A urinalysis and a serologic test for syphilis were negative. Skin tests for tuberculosis, coccidioidomycosis, histoplasmosis, and blastomycosis were negative. Throat smear and culture for pyogens showed no significant organisms. Sputum examination was negative for fungi on smear and culture. Culture of the bone marrow was negative for fungi. Initially the serum albumin was 3.5 gm. per 100 ml. and the globulin was 4.3 gm., but later determinations were within normal limits. The remainder of a group of liver function tests was normal. No lupus erythematosus cells were found in appropriate preparations. Several stool examinations were negative for ovae and parasites. Histologic examination of the bone marrow was normal.

The patient's course in the hospital was marked by normal vital signs. He continued to have a dry cough with some weakness and fatigue as well as repeated episodes of ulcer symptoms and attacks of chest pain due to spontaneous pneumothorax on either side (figure 1). Since a majority of the pneumothoraces were on the right side, a right thoracotomy was done in November, 1954. At operation a bizarre picture presented.

The lung was studded with numerous small grayish vesicles varying from approximately 5 to 10 mm. in diameter. Nodules of the same size were palpable throughout the lung. A wedge biopsy was done and the tissue was submitted for histologic examination and cultures for pyogens, fungi, and tubercle bacilli, as well as tissue cultures for viruses. The parietal pleura was abraded with gauze in order to promote adhesions. The patient recovered well from the operation and thereafter had no further episodes of spontaneous pneumothorax on that side. The above-enumerated studies of the lung tissue for microorganisms were negative.

Microscopic examination of serial sections of the entire specimen of pulmonary paren-

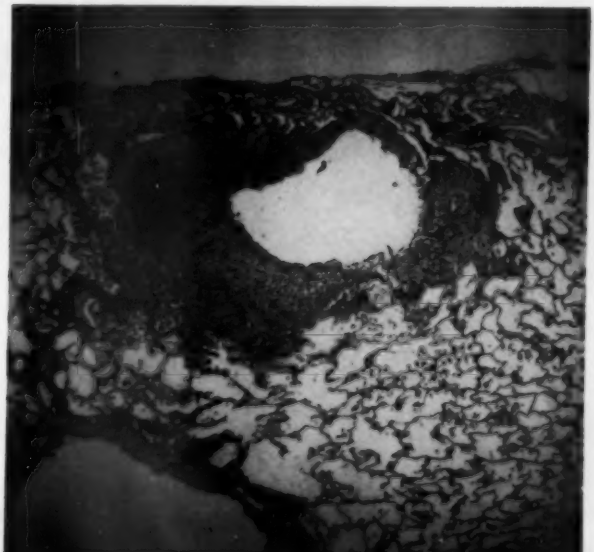

FIG. 3. Low-power microphotograph showing cystic subpleural granulomatous focus.

chyma revealed well-demarcated masses of oval and spindle cells resembling reticulum cells in which capillary vascularization was prominent at the periphery. Elongate and spindle-shaped cells resembling proliferating fibrous connective tissue provided a framework for this basic cell type. Centrally, there were intense accumulations of polymorphonuclear leukocytes and eosinophils with focal central necrosis and absence of material creating a hollowed defect (figure 3). There was an occasional multinucleated giant cell, and numerous macrophages were seen throughout this granulomatous tissue (figure 4). The macrophages within the lesion contained brown, granular pigment within the cytoplasm, and resembled those in adjacent, more normal appearing, alveoli. The basic cell type was one of poorly delineated cytoplasmic outline with fine cytoplasmic granularity and nuclei of variable size and shape which tended to be oval or elongate. Nuclear chromatin was finely and uniformly granular; hyperchromatism was not present. A rare mitotic figure was identified. While there were no constant morphologic relationships of the granulomatous lesions to blood vessels or to bronchi, the vessel walls, when involved by the granulomatous process, were infiltrated with inflammatory cells and were focally necrotic. Lesions of subpleural location were associated with focal fibrosis of the pleura and with infiltration by eosinophils and occasional polymorphonuclear leukocytes involving the pleural surface (figure 3). Such lesions provide an adequate explanation of the occurrence of spontaneous pneumothorax.

A diagnosis of eosinophilic granuloma was entertained, but a bone survey, including the skull, showed no lesions. An intravenous pyelogram was negative. An upper gastrointestinal series showed some deformity of the duodenal cap but no definite ulcer niche. Following the initial operation, the patient continued to have attacks of spontaneous pneumothorax on the left side. Therefore, in March, 1955, he had a left thoracotomy with exactly the same findings as were noted on the right side, grossly and microscopically. Again, abrasion of the parietal pleura was done and, following operation, the patient no longer had any attacks of pneumothorax. The induction miniature film of 1950 was obtained; it was normal.

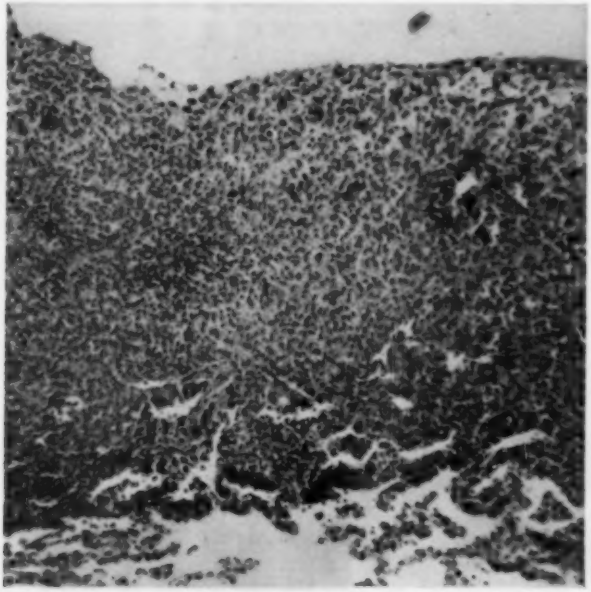

FIG. 4. High-power microphotograph of cystic lesion with luminal margin superior.

Additional studies included the following: serum amylase and glucose tolerance test at the time of greatest epigastric pain, cholecystogram, barium enema, renal function tests, 17-ketosteroid excretion, and electrophoretic pattern of serum globulin. These were all within normal limits. A sigmoidoscopy with biopsy of rectal mucosa for parasites was negative. Because of several recent reports of benefit from steroid therapy in eosinophilic granuloma, a three-week course of corticotropin and cortisone was given without change in the patient's condition. Also a course of roentgen therapy was tried in the amount of 1,000 roentgens as measured in air, given to the right lung over a period of two weeks. No changes were noted in the right lung as compared with the left lung.

The patient's symptoms continued at the same level and over a ten-month period of hospitalization he gradually lost twenty-one pounds in weight. Accordingly, he was separated from the Air Force in June, 1955. Soon thereafter he began to have polydipsia and polyuria and was admitted to a Veterans Administration hospital where routine studies were normal. However, his urine had a specific gravity of 1.001 to 1.003. Urine was negative for Bence-Jones protein. He excreted 6 to 9 liters of urine daily. Skull films were normal. Skin tests for tuberculosis were again negative. Intravenous hypertonic saline infusion failed to diminish the rate of urine flow. Pitressin® therapy resulted in the disappearance of his polyuria and a diagnosis of diabetes insipidus was made. The last observation of the patient was in February, 1956. No subsequent information has been obtained.

Discussion

In 1953, Lichtenstein (1) reviewed the nosologic entity which he called histiocytosis X and classified it into localized and disseminated forms. The former is localized to bone and is known as eosinophilic granuloma. It may present either solitary or multiple lesions. The disseminated forms include the acute or subacute Letterer-Siwe syndrome, with or without destructive bone lesions, and it sometimes becomes transformed into the chronic disseminated form or the Hand-Schüller-Christian syndrome. The latter may sometimes be associated with destructive bone lesions and early extraskeletal lesions resembling eosinophilic granuloma; it may undergo acute or subacute exacerbation similar to the Letterer-Siwe syndrome. Thus all sorts of clinical combinations may be seen. The localized disease and the chronic disseminated form tend to occur in children and young adults, whereas the acute disseminated disease tends to occur in infancy. However, any form may occur at any age, a few cases having been described in older adults.

Clinically, the acute disseminated form is insidious in onset and rapidly fatal. It is characterized by progressive weakness, weight loss, anemia, lymphadenopathy, hepatosplenomegaly, debilitation, and superimposed infection. Pathologically, the organs of the reticuloendothelial system are infiltrated with nonlipid histiocytes and similar infiltration may occur in other organs.

The chronic disseminated form was once characterized by Christian as a triad of exophthalmos, bone lesions, and diabetes insipidus. However, it is now obvious that the entire triad may not be present and other manifestations may occur, depending on the localization of lesions (skin, mucous membranes, et cetera). Pathologically, the disease is granulomatous and lesions are characterized by lipid-containing histiocytes, a few eosinophils, and fibrosis.

Eosinophilic granuloma of bone may occur alone or as part of the disseminated forms. Pulmonary involvement has also been reported as a solitary manifestation of histiocytosis X (7) but it is probable that sooner or later other organs become involved in such cases, as occurred in the case herein reported. The pulmonary disease is diffuse and nodular. Only a few recent cases have been subjected to biopsy in the early stage when the lesions resemble those of eosinophilic granuloma of bone and several cases have presented the cystic spaces which characterize the so-called "honeycomb lung." More commonly,

honeycombing has been noted only in the end-stage process present at autopsy when the granulomatous changes are largely replaced by fibrosis (9). "Honeycomb lung" is a nonspecific term as it has been described in tuberous sclerosis, bronchiectasis, and bronchiolitis. The pathogenesis of "honeycomb lung" in histiocytosis X is open to speculation. Cunningham and Parkinson (9) have suggested that the cystic spaces are due to weakening of the bronchiolar walls by the granulomatous infiltration. Keats and Crane (12) have suggested that the cystic spaces are due to central necrosis in the nodular granulomata followed by cystic expansion of the necrotic foci due to ingress of air and elastic contraction of adjacent normal parenchyma. It is of interest that similar cystic lesions have been recently described in a scalene node by Grant and Ginsburg (13) in a case very similar to the one described here.

Nothing is known about the etiology of this disease. It would seem likely that there is an infectious agent because of the inflammatory nature of the disease. However, all attempts to isolate a causative organism have failed. In the present case these attempts included tissue cultures for viruses but this also was unrewarding.

The prognosis for cure with roentgen therapy is good for localized bone lesions. The acute disseminated form of histiocytosis X is usually fatal, and the prognosis for chronic disseminated disease is guarded, death usually being due to severe pituitary damage or progressive pulmonary fibrosis, insufficiency, and cor pulmonale. There is no specific therapy for the disseminated disease, although a few cases have been reported with allegedly beneficial results in the pulmonary lesions after steroids or roentgen therapy.

Summary

A case of pulmonary histiocytosis X with "honeycomb lung," complicated by recurrent bilateral "spontaneous" pneumothorax and diabetes insipidus, has been described. The diagnosis was established by bilateral lung biopsies. The etiology of this disease was not established by bacterial and viral studies of the tissue.

Sumario

Histiocitosis X Pulmonar

Se describe un caso de histiocitosis X pulmonar con "pulmón en panal," complicado por neumotórax "espomtáneo" bilateral recurrente y diabetes insípida. El diagnóstico fué establecido por biopsias pulmonares bilaterales. La etiología de la dolencia no fué establecida por estudios bacteriológicos y virales del tejido.

Resume

Histiocytose pulmonaire X

Description d'un cas d'histiocytose X du poumon avec poumon "en nid d'abeilles" compliqué de pneumothorax "spontané" bilatéral récidivant et de diabète insipide. Le diagnostic fut établi à l'aide de biopsies pulmonaires bilatérales. L'étiologie de cette affection ne fut pas établie par l'étude bactériologique et virale des pièces.

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A CASE OF SUPERNUMERARY RIGHT BRONCHUS WITH ACCESSORY HYPOPLASTIC LOBE CONTAINING A BRONCHIAL ADENOMA[1]

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Introduction

Supernumerary bronchi are comparatively rare. According to some writers (1–5) they are most commonly seen in connection with the right upper lobe arising from the trachea and lateral or medial wall of the upper part of the main right bronchus. A case of supernumerary bronchus originating from the anterior wall of the lower part of the right main bronchus was recently encountered in the course of a routine post-mortem examination, presenting features which are considered to be of sufficient interest to warrant a report.

The patient was a 60-year-old white male who was admitted to Cumberland Hospital in shock and cyanosis and died one hour after admission. Necropsy disclosed the cause of death to be acute pancreatic necrosis.

The anomalous finding in the right lung (figures 1–5) consisted of a hypoplastic accessory lobe, 5 cm. in greatest dimension, emerging anteromedially from the lower part of the right main bronchus. The parenchyma was firm. The supernumerary bronchus arose anteriorly 4 cm. from the carina and its orifice was 1.2 cm. in diameter. The bronchus was 3 cm. in length and ended abruptly in lung tissue. Sections of the hypoplastic lung showed atelectatic parenchyma containing clusters of uniform, round and oval cells with poorly defined cytoplasm. Some of the cells formed cords or pseudoglands, embracing a congested network. The connective tissue formed thin strands which separated nests of epithelial cells (figure 6). The other three lobes of the right lung were normal (figures 1–5). The superior segmental bronchus of the right lower lobe in this case, as usual, divided into its three subsegmental bronchi—superior, medial, and lateral. The superior branch arose first, the remaining two arose by bifurcation (figures 3 and 4). The lungs were studied in an unfixed specimen in a collapsed condition and the bronchial tree was dissected.

A roentgenogram of the specimen taken after tracing the segmental bronchi (figure 5) shows the ten segmental normal bronchi and the nodular density produced by the accessory lobe with its supernumerary bronchus at the hilum of the right lung. This density must be differentiated from benign or malignant bronchogenic neoplasms (6), from localized saccular bronchiectasis with inspissated exudate (7), or from accessory lung arising from the esophagus (8). An ante-mortem chest film was not available for comparison.

Summary

An unusual case of a supernumerary bronchus arising from the anterior wall of the lower part of the main right bronchus, with the accessory lobe containing a benign adenoma, is described. The origin of this anomalous bronchus is traced to the lower part of the main right bronchus unlike the cases previously reported. A careful search of the literature failed to reveal any similar anomaly. This congenital anomaly must be borne in mind in differentiating nodular densities at the hilum of the right lung.

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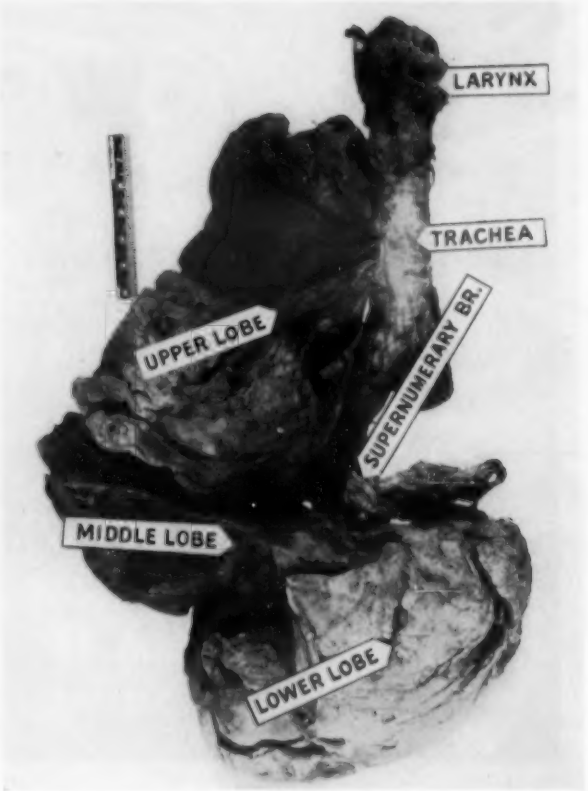

FIG. 1

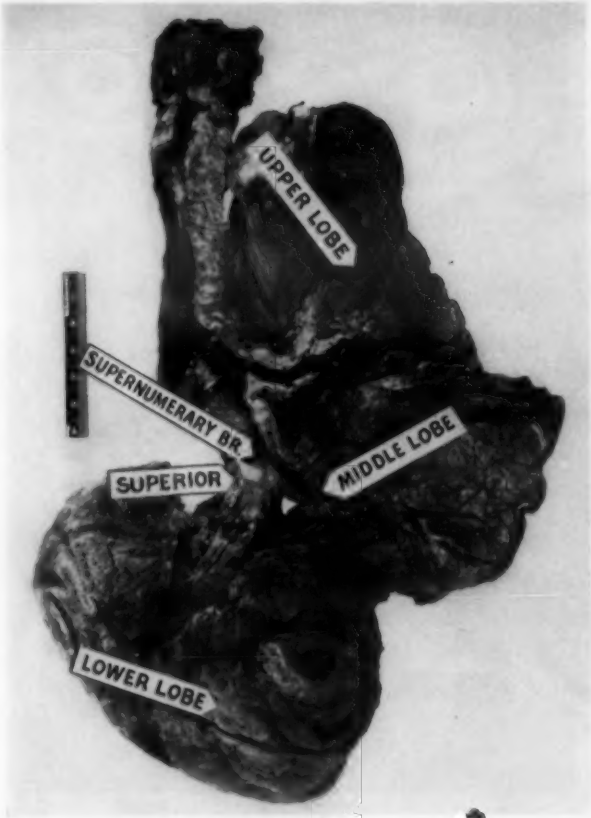

FIG. 2

FIG. 1. Anterior view of the right lung, showing the normal lobes and bronchi in relation to the supernumerary bronchus.
FIG. 2. Posterior view of the right lung showing the wide orifice of the supernumerary bronchus.

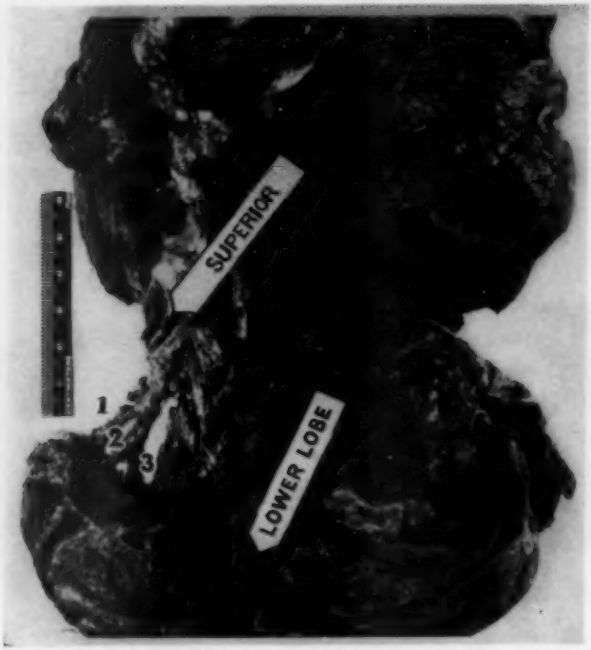

FIG. 3. The right lung, showing the superior bronchus of the lower lobe with its three divisions (*1*, *2*, and *3*).

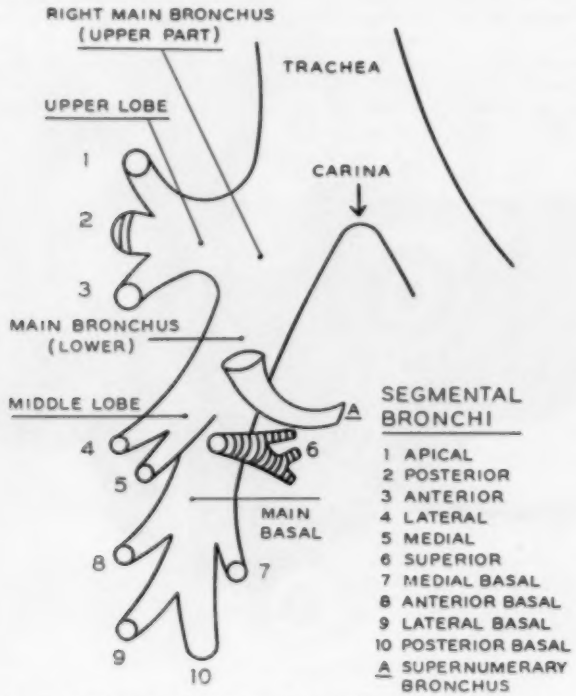

FIG. 4. Diagram of the bronchial tree of the right lung, showing the normal bronchi (*1–10*) and their relation to the supernumerary bronchus (*A*).

FIG. 5

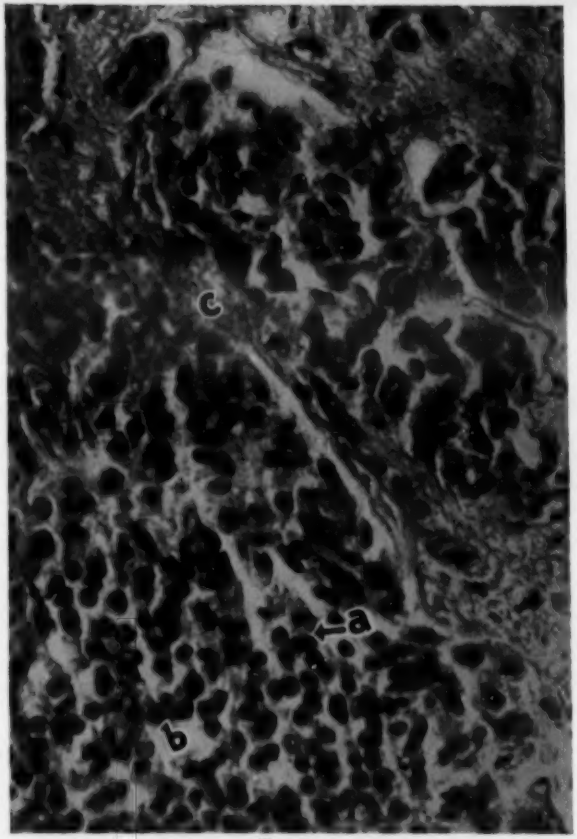

FIG. 6

FIG. 5. Roentgenogram of the anterior view of the right lung (post-mortem specimen). The ten segmental bronchi are indicated by wire probes labeled *1* through *10*. Note also the nodular density of the accessory lobe (*A*) and its supernumerary bronchus.

FIG. 6. Photomicrograph of the neoplasm in the supernumerary lobe showing the benign adenoma composed of round and oval cells and forming cords (*a*) and pseudoglands (*b*). Thin strands of connective tissue (*c*) separate nests of cells.

Sumario

Caso de Bronquio Derecho Supernumerario con Lóbulo Hipoplásico Accesorio que Contenía un Adenoma Bronquial

Se describe un caso extraño de bronquio supernumerario que partía de la pared anterior de la porción inferior del principal bronquio derecho, con un lóbulo accesorio que contenía un adenoma benigno. El origen de este bronquio anómalo se traza a la porción inferior del principal bronquio derecho, lo cual es distinto de lo observado en los casos ya comunicados. Un repaso cuidadoso de la literatura no reveló ninguna anomalía semejante. Hay que tener presente esta anomalía congénita al diferenciar condensaciones nodulares en el hilio del pulmón derecho.

Resume

Un cas de bronche droite surnuméraire avec lobe accessoire hypoplastique renfermant un adénome bronchique

Description d'un cas rare de bronche droite surnuméraire naissant de la paroi antérieure du segment inférieur de la bronche-souche droite avec lobe accessoire renfermant un adénome bénin. A l'encontre des cas antérieurement publiés, l'origine de cette bronche anormale est trouvée à la partie inférieure de la bronche-souche droite. De minutieuses recherches dans la littérature médicale n'ont révélé aucune anomalie semblable. Quand il s'agit de différencier les opacités nodulaires hilaires au niveau du poumon droit il y a lieu de penser à cette anomalie congénitale.

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Notes

RAPID DETECTION OF DRUG-RESISTANT TUBERCLE BACILLI IN SPUTUM BY SLIDE CULTURES

The methods currently employed for determination of drug resistance of tubercle bacilli in sputum are not entirely satisfactory. The most prominent deficiency of these methods is their lack of promptness. Tubercle bacilli, and especially some drug-resistant strains, grow so slowly on solid media that commonly a period of three to six weeks passes before the laboratory is able to submit results to the physician.

The time required for detection of growth of tubercle bacilli can be shortened appreciably by use of slide culture techniques. Since the report of Pryce[1] in 1941, many modifications of the method have been studied, some workers reporting favorable, and others unfavorable, results. Reed and Morgante[2] have recently summarized their own investigations and have reviewed extensively the literature dealing with slide cultures. This technique has been employed for drug-susceptibility determinations by foreign workers,[2-4] but has received little attention in the United States.

The present communication presents a comparison of results obtained by standard egg slant and by a slide culture method in performing drug-susceptibility tests on specimens from tuberculous patients.

Sputum samples were collected over a twenty-four-hour period from patients in the Rockefeller Institute Hospital. In the patients who received drug therapy, all antimicrobial agents were withheld for a period of twenty-four hours preceding the sputum collection, as well as during the twenty-four-hour collection period.

The slide culture tests were performed as follows: New glass slides (1 by 3 inches) for standard microscopic use were cut longitudinally into three strips. These strips (approximately one-third by 3 inches) were labeled at one end by etching with a diamond pencil and were then rinsed in distilled water and thoroughly cleaned with a linen towel. The clean slides were placed in groups of four in sterile Petri dishes (10 cm. in diameter). Small portions of the sputum sample were smeared over the slides and drying was accomplished at room temperature within thirty to sixty minutes. Six per cent sulfuric acid was then added to the Petri dishes so that the slides were completely immersed. After six minutes of exposure to acid, the slides were removed with forceps, held above a 10 per cent solution of Azeesol®, and washed thoroughly with sterile distilled water from a capillary pipette. When the draining fluid no longer produced a precipitate in the Azeesol, it was assumed that the washing was sufficient. Duplicate slides were then deposited back to back in sterile cotton-plugged Pyrex® test tubes (13 by 100 mm.) containing 5 ml. of medium.

The medium employed for slide cultures was the basal liquid medium containing oleic-acid–albumin complex as described by Dubos and Middlebrook.[5,6] Sterile aqueous so-

[1] Pryce, E. M.: J. Path. & Bact., 1941, *53*, 327.

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[6] The Dubos basal medium used contained 2.0 gm. asparagine, 0.5 gm. N-Z amine (type *B*), 0.05 ferric ammonium citrate per liter, and standard amounts of the other ingredients.

lutions of streptomycin,[7] isoniazid,[8, 9] or PAS,[10] were serially diluted in the sterile complete medium so as to produce final concentrations of the antimicrobial drugs listed in the tables.

The slide culture tubes were incubated in an upright position at 37°C. After incubation for five days, one of the slides was removed from the control tube containing no drug. After drying at room temperature, this slide was fixed with absolute methanol and stained for acid-fast bacilli by the standard Ziehl-Neelsen technique. (The staining with carbolfuchsin was carried out for ten minutes on a metal staining rack over a steam bath. Repeated attempts to culture tubercle bacilli after staining of heavily positive slide cultures have proved this treatment adequate to kill the organisms on the slide.) If multiplication of tubercle bacilli was observed on microscopic examination of this control slide, slides from the series of tubes containing antimicrobial drugs were removed, stained, and examined in like fashion. If no growth was evident on the slide from the control tube, the incubation was continued and all slides, including the duplicate in the control tube, were examined after a total of seven or more days of incubation.

After small portions of the sputum sample had been removed for the slide cultures, the remainder was incubated for 30 minutes at 37°C. with an equal volume of 3 per cent NaOH and occasionally agitated. The digested sputum was centrifuged (International Centrifuge, size 2) for 20 minutes at 2,500–3,000 r.p.m., the resultant supernatant was discarded, and the sediment was neutralized with 1N HCl in 0.1 per cent phenol red indicator, followed by N/10 NaOH or N/10 HCl as required. The sediment was then suspended in 40 ml. of 0.1 per cent sterile bovine albumin in distilled water and recentrifuged. The supernatant was discarded and the sediment inoculated onto egg slants.

Modified Löwenstein-Jensen egg medium,[11] distributed in screw-capped potato tubes, 15 ml. per tube, was freshly prepared before use. Drug-susceptibility tests were performed according to directions outlined in the revised bulletin of the Veterans Administration Conference on the Chemotherapy of Tuberculosis.[12] Appropriate amounts of streptomycin, isoniazid, or PAS were added to the egg medium before inspissation so as to produce after inspissation drug levels as indicated in table 3. The experience of the present writers with susceptibility studies performed simultaneously on other media suggests that the effective concentrations of streptomycin in the egg slants are often at least two- to fourfold lower than those indicated in table 3. After inoculation with sputum sediment, the egg slants were incubated at 37°C. and examined twice weekly for the growth of tubercle bacilli.

All procedures involving exposure to infectious material were carried out in hoods designed for the protection of laboratory personnel.

* * *

The sputum specimens which yielded growth on slide culture were positive for acid-fast bacilli on direct smear (*see* below). Therefore, it was essential to use as a criterion for a positive slide culture *multiplication* of tubercle bacilli, not their mere presence. Growth was usually readily recognizable by gross examination after staining. Photomicrographs

[7] Streptomycin sulfate, USP, from Eli Lilly and Co.

[8] From Nutritional Biochemicals Corp.

[9] The solutions of isoniazid and PAS were sterilized by passage through bacteriologic porcelain filters.

[10] Sodium para-aminosalicylate from Parke, Davis and Co.

[11] American Trudeau Society: Am. Rev. Tubere., 1952, *65*, 105.

[12] Hall, W. H., *et al.*: Diagnostic laboratory methods as revised following the Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, February, 1953.

TABLE 1

GROWTH OF TUBERCLE BACILLI FROM SPUTUM SPECIMENS POSITIVE BY DIRECT SMEAR

Patient	Date of Specimen	Time (in days) Required for Detection of Growth	
		Slide Culture	Egg Slant*
J. M.	11/17/55	5	19
	12/22/55	5	30
	1/12/56	5	40
Ju. M.	11/14/55	6	14
K. H.	11/17/55	3	19
B. B.	3/8/56	7	18
	4/19/56	10	32
C. T.	2/9/56	5	25
	4/5/56	7	26
	4/19/56	5	12
	5/3/56	5	17
B. N.	4/9/56	7	18
	4/20/56	6	11
E. T.	1/26/56	5	19
	2/9/56	5	33
J. Ma.	4/9/56	7	11
	4/19/56	5	12
	5/3/56	5	18
	5/17/56	5	18
	5/31/56	5	18
M. B.	3/15/56	5	19
	3/22/56	5	18
L. S.	2/2/56	5	34
	2/9/56	5	42
	2/23/56	5	21
	3/9/56	7	46
M. V.	2/23/56	5	21
	3/8/56	5	18
	3/15/56	5	21
	3/22/56	5	18
G. G.	1/26/56	10	26

* More than 100 colonies or confluent growth on all slants.

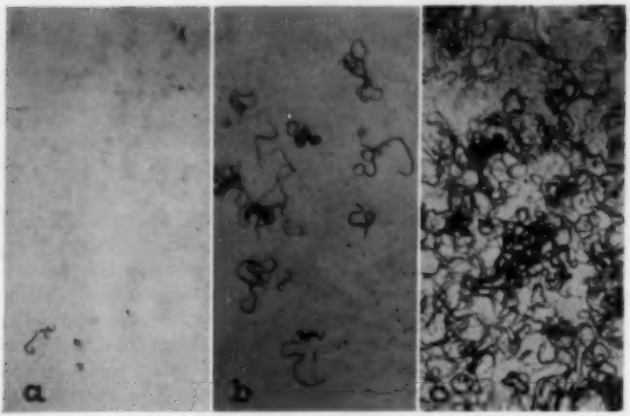

FIG. 1: Photomicrographs of slide cultures of sputum from three different patients. Incubated seven days at 37°C. (Ziehl-Neelsen stain, × 124)

TABLE 2

GROWTH OF TUBERCLE BACILLI FROM SPUTUM SPECIMENS NEGATIVE BY DIRECT SMEAR

Patient	Date of Specimen	Slide Culture		Egg Slant	
		Time of Observation	Growth	Time of Detected Growth	Number of Colonies per Slant
		days		*days*	
G. G.	1/9/56	8	Negative	17	2 3 5
	2/9/56	5	Negative	33	1 1 0
	2/23/56	5	Negative	41	1 0 1
M. B.	1/12/56	5	Negative	58	1 0 0
	4/19/56	5	Negative	50	9 7 0
C. T.	2/23/56	5	Negative	22	>100
J. M.	2/23/56	5	Negative	21	1 0 0
E. T.	2/23/56	5	Negative	23	8 3 5
B. B.	4/6/56	5	Negative	25	10 2 4
B. B.	4/19/56	11	Positive	32	6 0 0

illustrating the appearance of serpentine microcolonies under low power magnification (× 124) are presented in figure 1. The illustrations show slide cultures in which only a few microcolonies have appeared (*a*), one containing moderate numbers of colonies (*b*), and a heavily positive slide culture (*c*). Examination under oil immersion, although seldom necessary, served to distinguish between isolated bacilli or small clumps which might have been present on inoculation and serpentine microcolonies characteristic of *in vitro* multiplication.

A comparison of slide cultures and egg slants for routine cultivation of tubercle bacilli from sputum is presented in tables 1 and 2. For purposes of clarity, the specimens are divided into those positive for acid-fast bacilli by smear (table 1) and those in which no tubercle bacilli were seen in a 30-minute search of a direct smear stained by the Ziehl-

TABLE 3

Resistance Assays of Tubercle Bacilli

Patient	Date of Specimen	Time (in days) Required for Detection of Growth																
		Slide Culture											Egg Slants					
		Control	Drug (γ/ml.)										Control	Drug (γ/ml.)				
		No Drug	Isoniazid				Streptomycin				PAS		No Drug	Isoniazid		Streptomycin		PAS	
		0	1	5	25	125	1	5	25	125	1	10	0	1	5	10	100	1	10
M. B.	3/15/56	5*	5	5	5	—†	—	—	—	—	—	—	19	19	19	—	—	—	—
	3/22/56	5	ND‡	ND	5	—	—	—	—	—	ND	ND	18	18	18	—	—	—	—
	5/16/56	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
M. V.	3/8/56	5	—	—	—	—	5	5	5	—	—	—	18	—	—	18	18	—	—
	3/15/56	5	—	—	—	—	5	5	5	—	—	—	21	—	—	21	21	—	—
	3/22/56	5	—	—	—	—	5	5	5	—	—	—	18	—	—	23	23	—	—
L. S.	3/9/56	7	7	7	7	—	7	7	7	7	ND	ND	46	46	46	46	46	ND	ND
B. N.	4/20/56	6	6	6	6	—	—	—	—	—	—	—	11	30	30	—	—	—	—
C. T.	4/5/56	7	—	—	—	—	7	—	—	—	—	—	26	—	—	33	—	—	—
	4/19/56	5	—	5	—	—	5	5	—	—	—	—	12	40	—	53	—	—	—
	5/3/56	5	—	—	—	—	5	5	—	—	—	—	17	—	26	38	—	—	—
	5/17/56	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
J. Ma.	5/17/56	5	5	—	—	—	—	—	—	—	—	—	18	Cont.	—	—	—	—	—
	5/31/56	5	—	5	—	—	—	—	—	—	—	—	18	—	—	—	—	—	—
J. L.	9/21/56	5	—	—	—	ND	—	—	—	ND	ND	ND	19	—	—	—	—	ND	ND

* Number of days required for detection of growth.
† Negative (no multiplication).
‡ Not done.

Neelsen procedure (table 2). When the sputum contained reasonably large numbers of tubercle bacilli, both slide and egg slant cultures were uniformly positive, as is shown in table 2. In confirmation of reports of many other workers,[2] growth was observed at a much earlier date by slide culture than by egg slant culture. In the small series of samples in the present report, growth was usually detected by slide culture after five days of incubation, while the egg slants required an average of twenty-two days before colonies were visible. Moreover, in the case of one patient, *L. S.*, whose organisms on repeated occasions required a long incubation period on egg slants, growth was obtained within five days by means of slide culture.

The findings on those sputum samples which contained only small numbers of tubercle bacilli (negative by smear) are summarized in table 2. It may be seen that the slide cultures were negative after one week of incubation, while the standard egg slants (inoculated in triplicate for each specimen) yielded small numbers of colonies on prolonged incubation. It is important to point out that these results do not necessarily indicate that slide cultures are less sensitive than egg slants; the sample inoculated onto the slide was many times smaller than that used on the slants, and in addition the material placed on slants had been concentrated while that used for slides had not.

A comparison of drug-susceptibility studies on 15 sputum specimens in slide cultures and on egg slants is presented in table 3. It is seen that the results, in terms of resistance or susceptibility to various concentrations of streptomycin, isoniazid, or PAS, were essentially the same by the two methods. The difference in time required for the determination, however, was quite striking, the results being available by slide culture within one week while those on egg slants required from three to more than six weeks.

* * *

A rapid method for the determination of resistance of tubercle bacilli to antimicrobial agents is becoming more urgent with the widespread use of streptomycin, isoniazid, PAS, and other agents in the therapy of tuberculosis. As in the case of other infectious diseases treated with antimicrobial agents, prolonged therapy often results in the emergence of organisms which have lost their susceptibility to the specific bacteriostatic or bactericidal agent employed. Rapid detection of resistance may become more important in the future if primary infection with drug-resistant strains is more frequently encountered.

The results obtained in this study confirm many previous reports that growth of tubercle bacilli can be recognized within a period of one week by slide cultures of sputum, and serve to endorse the usefulness of the slide culture technique for early detection of resistance to antimicrobial agents.

The slide culture has two decided advantages over the egg slant culture in relation to susceptibility tests: (*1*) the rapidity with which results are available makes it possible for the physician to know the drug susceptibility of the tubercle bacilli before starting therapy on a newly diagnosed case, and enables him to follow the course of therapy in a more satisfactory manner, and (*2*) it seems likely that the slide cultures will prove more accurate than egg slants, since the slide method avoids inspissation and prolonged incubation, both of which may lead to destruction of the antimicrobial agents.

The present experience suggests that slide cultures are satisfactory for resistance tests only on sputum specimens which contain tubercle bacilli detectable by microscopic observation of a stained direct smear. This limitation is not so severe as it may seem at first glance; in precisely those clinical situations in which resistance studies are most urgently needed, e.g., at the start of therapy or when the course is unfavorable, frequently sputum specimens are heavily positive for acid-fast bacilli.

Further studies of slide cultures now in progress in many laboratories may well lead to

technical improvements. The procedure of picking samples from untreated sputum for slide cultures is not an ideal one. A suitable means of digesting and concentrating sputum for slide cultures would increase the sensitivity of the technique and would yield a more representative sample of bacilli from various parts of the sputum.

In summary: In confirmation of many reports, growth of tubercle bacilli has been detected within one week or less by means of slide cultures of sputum from tuberculous patients.

A slide culture and an egg slant technique have been compared for assaying susceptibility of tubercle bacilli to antimicrobial agents. On sputum specimens positive for acid-fast bacteria by direct smear, drug resistance of tubercle bacilli can be accurately determined within one week by slide cultures in contrast to the prolonged incubation period required on egg media.

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October 24, 1956

EVALUATION OF TRITON MALACHITE GREEN CHARCOAL AGAR MEDIUM FOR DETECTION OF TUBERCLE BACILLI[1]

A simply prepared inexpensive charcoal agar medium for culturing tubercle bacilli was described by Hirsch in 1954.[2] Results with laboratory strains of *M. tuberculosis*, inoculated from culture dilutions and from ground animal organs after experimental infection, suggested that it might be useful for diagnostic purposes. Experiments in this laboratory have shown, however, that the new medium is less efficient than that prepared from the modified Petragnani formula employed routinely in this laboratory. This conclusion was reached after more than 1,000 sputum and other specimens were used for comparative tests. A total of 155 of these was found to contain living bacilli when cultured on either medium; but there were 80 instances when only the routine medium detected organisms and the new one did not. The converse occurred on but two occasions.

TABLE 1

COMPARISON OF CULTURAL RESULTS FOLLOWING INOCULATION OF MODIFIED PETRAGNANI SLOPES AND TRITON MALACHITE GREEN CHARCOAL AGAR MEDIUM WITH NEUTRALIZED SEDIMENT FROM HOMOGENIZED SPUTUM AND OTHER SPECIMENS

Results of Culturing		Number of Cultures
Modified Petragnani Medium	Charcoal Medium	
−	−	865
+	+	152
+	−	45
−	+	12
+	Contaminated	1
−	Contaminated	3
Contaminated	−	2
Contaminated	+	1
		Total 1,081

More recently, Hirsch[3] has developed a modification of his original charcoal medium. This was thought to be superior in producing growth from small inocula of certain strains. The new formula differs only in that it contains triton WR 1339, which is a polyoxyethylene ether, and malachite green. Comparisons performed in this laboratory have indicated that the modified medium is a better one than that prepared from the original formula, but still not quite as good as the inspissated egg-potato medium in routine use. The difference is slight and may not, indeed, be significant. The results are shown in table 1.

Both of the charcoal media suffer from the disadvantage that the slants must be transported, incubated, and stored in the horizontal position. Colony morphology with the triton malachite green modification is quite different from that with which technicians handling routine diagnostic work are likely to be accustomed, the colonies being mucoid and smoother. Also the charcoal media are not suitable for isoniazid susceptibility testing.[4]

[1] This investigation was supported by a research grant from the Department of National Health and Welfare.

[2] Hirsch, J. G.: Am. Rev. Tubere., 1954, *70*, 955.

[3] Hirsch, J. G.: Am. Rev. Tubere., 1955, *71*, 894.

[4] Lewin, E., and Hirsch, J. G.: Am. Rev. Tubere., 1955, *71*, 447.

Against these disadvantages, ease of preparation, reproducibility, ready availability, inexpensiveness, and stability on autoclaving are attractive assets. The modified formula may be useful for diagnostic work under certain circumstances.

In summary: Comparative tests were made with the triton malachite green charcoal agar medium of Hirsch and a modification of an inspissated egg-potato medium in common use. Although the charcoal medium has several advantages which might make it the medium of choice in certain circumstances, it appeared to be slightly less sensitive for diagnostic culturing of *M. tuberculosis*.

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CANADA

A. RILEY ARMSTRONG

August 22, 1956

CHRONIC BRONCHITIS: SOME CLINICAL, PATHOLOGIC, AND BACTERIOLOGIC ASPECTS[1]

During the past six years a weekly outpatient clinic has been held at the Brompton Hospital for bronchitic patients, at which some 2,000 new patients have been seen. Since 1952 the bacteriologic aspects have been studied by Dr. J. Robert May and the pathologic aspects by Dr. Lynne Reid.

No satisfactory definition of chronic bronchitis has ever been devised, but experience has shown that patients fall into a fairly well-defined pattern. The various phases through which bronchitic patients may pass are presented in table 1. Rather more than half develop their symptoms insidiously, the common story being that a cold, which apparently differed in no way from previous colds, descended to the chest, causing a cough and sputum for a week or two without obvious disability. For some reason which is not clear, these patients found that subsequent colds tended to go down to the chest. Presumably this complication of an upper respiratory infection represents a mild bronchitis, but the term bronchitis is not ordinarily used unless some disability exists either from the infection, or breathlessness, or both. A proportion of patients having colds which descend to the chest reach the stage of recurrent bronchitis in which respiratory infections become a regular feature of the winter months. Fully developed chronic bronchitis represents a state in which there is a permanent feeling of "chestiness" in the winter months or all the year round, with superimposed exacerbations of infection. When the onset is acute, patients may pass directly to the mild, recurrent, or chronic stage. Bronchospasm may be an added complication at any stage and was present at the time of interview in about two-thirds of the patients. Emphysema, a disease of uncertain etiology in some patients, is a constant feature of bronchitis provided the infective factor is prominent.

The macroscopic features of the sputum in 1,000 patients who visit the clinic are shown in table 2. Perhaps the most obvious feature is the large number of patients showing a mucoid sputum, unless there is actually an exacerbation present, in which case the majority show the purulent component. A few patients stated that they had no sputum at any time. This statement was made primarily by women or possibly in instances of so-called pure emphysema. The sputum was purulent at all times in only 14 per cent. By combining the features listed in table 1 with the character of the sputum, a fairly accurate classification of bronchitic patients can be obtained.

In considering the pathologic aspects, attention can be focused either upon the excessive formation of mucus or the purely infective factor. Mucus, with considerable proliferation of goblet cells in the bronchioles, is the most obvious change in the early stages, and the excessive mucus almost certainly has a resistance-lowering effect upon the bacteria present. Dr. Lynne Reid has paid particular attention to the infective aspects and, by serial sections, has demonstrated that the pathologic changes occur in their most destructive form in the bronchioles and primary lobules. Some of these changes, such as lobular pneumonia and atelectasis, are potentially reversible. Others, such as lobular and bronchiolar fibrosis, are necessarily permanent. Repeated infections seem to lead to progressive fibrotic changes, the more normal parts of the lung becoming dilated and having all the appearances of emphysema. Indeed, in chronic bronchitis, emphysema seems to be an inevitable accompaniment of lobular fibrosis, although the exact mechanism of development of the emphysema, as by compensation or a valvular mechanism, is not certain.

[1] Presented, in part, before the Medical Session of the annual meeting of the National Tuberculosis Association, New York, New York, May 21, 1956.

TABLE 1

THE CLINICAL PATTERN OF CHRONIC BRONCHITIS

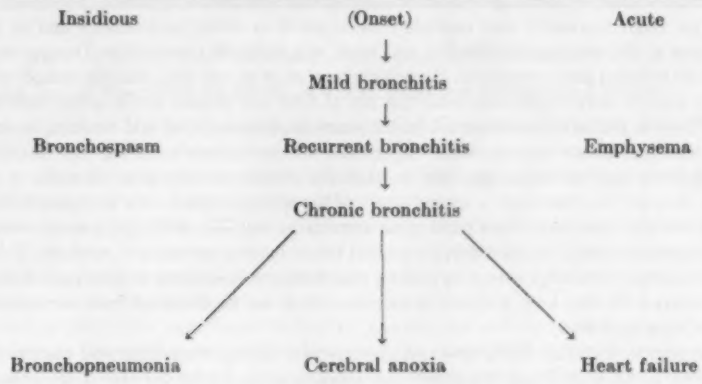

TABLE 2

CHARACTER OF THE SPUTUM IN 1,000 PATIENTS WITH CHRONIC BRONCHITIS

	In Summer	In Winter	During Exacerbations
None	306	60	41
Mucus	547	669	350
Muco-pus	147	271	609

TABLE 3

PREVALENCE OF PATHOGENIC BACTERIA IN THE SPUTUM OF CHRONIC BRONCHITIC PATIENTS

	Pus or Muco-pus	Mucus
	per cent	*per cent*
Remissions		
H. influenzae	80–90	10
Pneumococcus	20	5
Exacerbations		
H. influenzae	55–60	
Pneumococcus	50–55	

When pus is present, *H. influenzae* or pneumococcus can always be found.

Bronchographic studies confirm the pathologic changes already mentioned, especially in relation to bronchiolar obliteration. Indeed, the state of the lungs can often be gauged fairly accurately by the degree of satisfactory bronchiolar filling.

* * *

From the clinical standpoint, bronchitis is certainly a formidable disease in Britain. It is listed upon death certificates as causing approximately 30,000 deaths a year, which is 7 per cent of the total deaths; the morbidity figure is also about 7 per cent for the working population. Figures for other respiratory diseases are pneumonia 21,000, cancer 16,000, and tuberculosis 8,000.

Some factors undoubtedly contribute to the development of chronic bronchitis. A family history of bronchitis is approximately three times as common as in controls. Analysis of the disease by social grades shows clearly that the disease is closely associated with poverty. High morbidity and mortality rates occur in dusty occupations and in those members of the community who live and work in a polluted atmosphere. Dampness, fog, and cold weather also contribute. Bronchitis may start at any age, but the symptoms are nearly always more formidable after the age of fifty and deaths rarely occur before this age. There is probably no difference in the sexes in persons living and working in similar environments. However, men smoke more than women and are more exposed to polluted atmospheres and inclement weather, so that the disease is commoner in men.

Dr. Robert May has made a special study of the sputum with a view to trying to decide which are the most important pathogenic organisms. At first, difficulty was encountered in trying to obtain a representative bacterial count from a specimen of sputum. This has been overcome to a large extent by adding pancreatin to specimens of sputum before they are cultured. In this way, a much more even count can be obtained from several pieces of the same specimen.

The prevalence of *H. influenzae* and pneumococci during remissions and exacerbations is shown in table 3. These organisms are considered to be by far the most important pathogens in bronchitic patients in London. This conclusion has been arrived at by examining sputum before and after various antimicrobial drugs have been administered, noting which organisms are related to the presence or absence of pus.

In considering both short-term and long-term treatment, particular attention has been paid to patients having pus in the sputum. Exacerbations have responded most favorably to a combination of penicillin and streptomycin, the latter drug being particularly useful for the eradication of *H. influenzae*. Long-term trials of tetracycline are in progress and suggest that this drug has much to offer to some patients, especially in the winter months.

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Neville Oswald

July 11, 1956

Correspondence

LETHAL ALLERGIC SHOCK IN EXPERIMENTAL TUBERCULOSIS UNDER STREPTOMYCIN THERAPY

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

The statements of Choremis and associates[1] about the temporary new elevation of temperature along with changes in the roentgenogram during the isoniazid and streptomycin treatment of acute primary tuberculosis in children, and the subsequent considerations of Kitazawa,[2] have suggested this letter.

Several years ago, in Lima, Peru, trying to get some experience in experimental tuberculosis and streptomycin therapy, we observed a phenomenon which at first we were unable to explain. Our studies gave us the opportunity of writing a paper which we presented as a thesis to the Faculty of Medicine of San Marcos University in Lima. The paper was published in 1951.[3] We inoculated 14 healthy guinea pigs with virulent human tubercle bacilli from a twenty-day-old culture developed in potato broth-egg yolk medium, the strain having been obtained from a non-treated tuberculous patient. The inoculum, given subcutaneously at the level of one of the groins, consisted of 0.1 mg. of the culture. Drs. Julio Morales S. and César Ruiz C. from the Department of Bacteriology of the "Dos de Mayo" Hospital in Lima did the culture and prepared the inocula. Twelve animals were treated with streptomycin (7.5 mg. or 8 mg. daily) and 2 remained as controls. The treatment started approximately one month after the inoculation and lasted forty-five days in 2 animals and two months and eleven days in the remaining 10 animals.

The average time of life after the infection was three months and eight days in the control animals and three months and sixteen days in the treated group. In the latter, 3 animals died during the first week of treatment, namely, on the second, fourth, and seventh days, respectively.

At the time of autopsy, the animals which were used as controls revealed extensive exudative lesions as well as abundant tubercle bacilli. In the lungs of one of them we obtained an average of 1,204 tubercle bacilli per field. The guinea pigs which died within the first three months (this lapse corresponding in general terms to the period of therapy) showed a distinct arrest and regression of the lesions, especially in the lungs and liver, and a striking reduction and even absence of bacilli. In contrast, the animals which died after the three months disclosed, simultaneously, regressive and evolutive lesions, as well as a concomitant bacillary increase, fundamentally in the lungs. Six guinea pigs died within the first three months and 6 after this period of time; 3 animals died during the first week of therapy in the former group. "Blue forms," i.e., nonacid-fast forms with the morphology of tubercle bacilli, were found in the spleen and liver of one animal and in the spleens of 2 others.

The 3 animals which died unexpectedly at the beginning of therapy disclosed a singular histopathologic picture. In the lungs, spleens, and livers we found large masses of epithelioid cells containing necrobiotic zones and accumulations of neutrophils, macrophages, and lymphoid elements; the Langhans' giant cells were very scanty, and there was marked recent hyperemia between the epithelioid cell masses. Endarteritic lesions with fibrinous thrombi and a notable hemorrhagic alveolitis were also seen in the lungs, as well as hemorrhagic areas in the liver and within the masses of epithelioid cells in the lungs. In one of the

[1] Choremis, C. B., *et al.*: Am. Rev. Tuberc., 1955, *72*, 527.
[2] Kitazawa, Y.: Am. Rev. Tuberc., 1956, *74*, 155.
[3] Montes, M.: Arch. peruanos pat. y clin., 1951, *5*, 145.

animals a giant pulmonary hemorrhagic focus was noted. Even the bronchi were surrounded and invaded by the luxuriant epithelioid cell proliferation. A great number of hemosiderin-laden phagocytic reticuloendothelial cells were seen in the spleens. Tubercle bacilli were observed only in the lungs and spleens and at the level of the necrobiotic zones and accumulations of neutrophils, macrophages, and lymphoid elements. We obtained an average of 130 tubercle bacilli per field in the lungs and 17 in the spleens. The bacilli were very thin, long, markedly hypochromic and showed, frequently, a prominent dark granule at the center and/or at one or both ends. Very often, bacillary shadows, free blue granules, acid-fast corpuscles, and degenerating acid-fast rods were noted. Only occasionally acid-fast bacilli were noted within the epithelioid cells. Bacillary clumps were not observed. The bacillary changes were particularly marked in the lungs. We considered that there was an evident intense bacillary destruction because of the fact that our findings were similar to those indicated by Canetti[4] at the completeness of the caseification and which for this author are the testimony of the "direct bacillary destruction." We did not observe the granulo-adipose cells found by Levaditi and Vaisman[5] in the tuberculosis of the animals which, according to these authors, would have a fundamental role in the bacillary destruction. The rapid death of the animals, the notable perifocal inflammation, and the intense bacillary destruction led us to think of a grave endogenous tuberculin reaction, if we take the terminology of Canetti.[6] Evidently, we were dealing with hyperergic animals.

Our presumptions found support in statements of Rich and Schwartz. Rich[7] has indicated that in hyperergic animals the tubercle formation is accelerated and that in them, not only epithelioid cells are noted, but also elements of acute inflammation and even small abscesses. Besides, Schwartz (cited by Canetti[6]), working with allergic rabbits which he reinfected, observed that most of the animals died in shock within the first two or three days following the reinfection, and he found in the lungs, aside from congestive changes in the livers and spleens, neutrophilic and lymphocytic reaction, hemorrhagic suffusions, alveolar edema, and venous lesions with fibrinous thrombi. In our cases, in which the assertions of Rich and Schwartz were applicable, the massive epithelioid cell proliferation was surprising. We thought that the antibiotic had put the animals in optimal condition in order that the mononuclear phagocytes exercise their bacillary lytic power, which would bring about the conversion of those elements into epithelioid cells. Canetti[6] has stated that the price of the lethal activity of the mononuclear phagocytes upon the mycobacterium is their conversion into epithelioid cells and that the rate or speed of appearance of the latter in a tuberculous focus is directly influenced by the rate or speed of bacillary destruction. In our evidently hyperergic animals, it is possible that the liberated tuberculoprotein, due to the intense bacillary destruction, brought about, by its contact with the hyperergic tissues, the indicated tuberculin reaction, which was of sufficient severity to prove fatal. For us, then, the phenomenon was a lethal allergic shock subsequent to the streptomycin therapy. This was our interpretation at that time.

Department of Pathology
St. Francis Hospital
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August 30, 1956

Mario Montes

[4] Canetti, G.: El Bacilo de Koch en la Lesión Tuberculosa del Pulmón, Argos S. A. Editorial C. e I., Buenos Aires, 1948.

[5] Levaditi, C., and Vaisman, A.: Rev. de la tubere., 1949, *13*, 232.

[6] Canetti, G.: La Alergia Tuberculosa en el Hombre, Argos S. A. Editorial C. e I., Buenos Aires, 1948.

[7] Rich, A.: Patogenia de la Tuberculosis, Editorial "Alfa," Buenos Aires, 1946.

THE IMPORTANCE OF THE SOCIAL SCIENCES FOR THE CONTROL OF TUBERCULOSIS IN UNDERDEVELOPED AREAS OF THE WORLD

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

The plan for creation of hospital colonies for the treatment of tuberculous patients at a reduced cost in countries of limited means approved by the Mexican National Security Administration and commented upon by Dr. Leo Eloesser in the March, 1956, issue of the REVIEW deserves trials. Dr. Eloesser rightly points out its many advantages.

The settlement of such colonies ought to be preceded everywhere by the creation of a blueprint for an integrated evaluation at regular intervals of their many-sided consequences and effects. It is of fundamental importance that other aspects besides the economic one be considered.

No consideration has been given until now to the local peculiarities which in underdeveloped areas of the world interfere with official endeavors for the control of tuberculosis. On the contrary, uniform methods have been used without regard to their adequacy in each particular setting. The peculiar social traits of the communities from which families would be drawn to form the hospital colonies should receive everywhere earnest consideration before implementation of this type of segregation.

In certain towns of Southeast Asia, where survival is the most obvious problem for the average family, attempts to resettle families in the outskirts under far better living conditions than in the narrow circle within which they have grown have been notoriously unsuccessful in spite of grants of land, houses, et cetera, from governments. Every family is identified there by certain religious neighborhoods, by proximity to kinship groups, as well as by definite types of economic activities. In other countries, as in South America, the stigma attached to tuberculosis, social competition, and mobility would make such efforts for segregation unsuccessful. Many other examples could be given. Racial and cultural heterogeneity would present a serious obstacle. In certain underdeveloped areas, segregation may represent to an individual or to a family a far greater hardship than chronic disease and a curtailed life.

In the plan adopted by the Mexican National Security Administration, the social sciences, *in particular anthropology and social psychology*, have a very important place. Certain concepts and methods of these disciplines, in fact, should be all important in any plans for the control of causes of morbidity and mortality within any given population. We are not yet fully conscious perhaps of their importance for the determination of the human factors which interplay in the processes leading to disease and its cure.

As examples of some basic fields for research in every society in which plans for the control of tuberculosis are under way, the respective *roles* played by the physician and by the patient in their mutual relationships ought to be defined. The meaning of the disease for the common people should be ascertained, the desire to heal ought to exist among the patients treated or segregated, the adequate means of motivation to conformity with official endeavors should be known and used.

In summary: Progress in the social sciences requires that today efforts for the control of tuberculosis be preceded in the underdeveloped areas of the world by adequately integrated surveys of the whole *situation* in which the disease is transmitted. By temperament and by habit, administrators are fond of buildings and physical structures which demonstrate investment; but without basic objective knowledge of the people, of the spirit and structure of the society to which they belong, the buildings may remain deserted, and

costly physical structures with all the niceties of modern science may be inadequate. That knowledge is today within our reach.

TALLBACKEN
ALINGSÅS, SWEDEN

C. VIJIL TARDON

September 12, 1956

STREPTOMYCIN AND ISONIAZID RESISTANCE

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

Chaves and associates[1] have presented drug-susceptibility data on tubercle bacilli isolated from 663 individuals in the New York area who had not previously received chemotherapy for their tuberculosis. They defined "significant resistance" to isoniazid as any growth on 5.0 γ of isoniazid per ml. of medium, or growth on 1.0 γ of isoniazid per ml. equivalent to that on the control culture; "significant resistance" to streptomycin was defined as any growth on 1,000 γ of streptomycin per ml. of medium, or growth on 100 γ of streptomycin per ml. equivalent to that on the control culture. Using these criteria, the authors found 3 persons with primary resistance to streptomycin, 5 with primary resistance to isoniazid, and none with primary resistance to both of these drugs. This is a 1.2 per cent incidence of primary drug resistance by their definition, and is regarded by them as of little clinical significance.

The relationship of *in vitro* to *in vivo* resistance to streptomycin was studied at Trudeau Sanatorium, but has not previously been recorded. There were 135 patients who had received 42- to 120-day courses of daily streptomycin therapy between 1947 and 1949 and whose organisms were tested for susceptibility before they received daily streptomycin re-treatment in 1949 and 1950. "Good" clinical response was defined as reversal of infectiousness during treatment plus significant roentgenographic improvement. All patients were receiving rest therapy and a few, temporary collapse as well. There were 43 with far advanced, 85 with moderately advanced, and 6 with minimal pulmonary tuberculosis.

IN VITRO VERSUS IN VIVO STREPTOMYCIN SUSCEPTIBILITY

Susceptibility Status Prior to Streptomycin Re-Treatment	Number of Patients	"Good" Clinical Response to 3 or 4 Months of Streptomycin Re-Treatment
		per cent
All organisms susceptible to 1.0 γ of streptomycin per ml.	48	78
Some organisms resistant to 1.0, 2.5, or 5 γ but all susceptible to 10 γ of streptomycin per ml.	63	26
Some or all organisms resistant to 10 to 1,000 γ of streptomycin per ml.	23	24
	134	

From these data it was concluded that appreciable growth (more than a few colonies) on 2.5 or more γ of streptomycin per ml. of medium is apt to be associated with significant impairment of clinical response to continued streptomycin therapy. The occasional ex-

[1] CHAVES, A., *et al.*: Am. Rev. Tubere., 1956, *74*, 293.

ception to this observation is probably attributable in part to wide variations in the proportion of streptomycin-resistant to streptomycin-susceptible organisms in the host.

Others agree that loss of clinical response to streptomycin is more closely correlated with the appearance of growth on 2.0[2, 3] to 3.5 γ[4] than upon 10 or more γ per ml. of medium. Furthermore, these same investigators[2, 3, 4] believe that clinical resistance to isoniazid, although of less clinical importance than streptomycin resistance,[2] is apt to appear after growth occurs on 0.2–0.5 γ of isoniazid per ml. of medium, rather than upon 1.0 or more γ per ml. of medium.

In the report of Chaves and associates there were 32 persons with organisms showing some growth on 10 γ of streptomycin per ml. and 35 with some growth on 0.1 γ of isoniazid per ml. in addition to the 8 persons with "significant resistance." Many, if not all, of the 32 with organisms which grew on 10 γ of streptomycin per ml. may have shown considerable growth on 2.0–3.5 γ of streptomycin per ml. and hence may be anticipated to have shown a poor response to streptomycin therapy.

In addition, some of the 35 persons with organisms growing on 0.1 γ of isoniazid per ml. may have shown significant growth on 0.2–0.5 γ but not upon 1.0 γ of isoniazid per ml.; it therefore appears likely that the response to isoniazid therapy in some of these 35 would be less than in those persons with complete isoniazid susceptibility to 0.1 γ per ml.

In summary, the true incidence of primary streptomycin resistance, and possibly of primary isoniazid resistance as well, of a clinically significant degree in these 663 persons may be considerably higher than 1.2 per cent and may represent a warning.

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ROGER S. MITCHELL

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WILLIAM STEENKEN, JR.

October 30, 1956

THE RELATIONSHIP OF MYCOBACTERIA AND MAMMALIAN CELLS IN TISSUE CULTURE

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

Since 1953 a study has been in progress on the nature of the infective process of mycobacterium in tissue culture. Human, avian, and various animal tissues have been studied, the most stable tissue culture cell strains being two human epidermoid carcinomas, a human sarcoma,[1-4] and a mouse skin cancer. All of these grow well in human, horse, and bovine media. Guinea pig cell strains seem the most difficult to maintain, especially when grown in homologous serum. Cells were grown in clots or as suspensions; and the areal growth, phagocytosis, and cell (cell count) multiplication have been studied.

The relationship of mycobacteria and mammalian cells in tissue culture was studied,

[2] RUSSELL, W. F., JR., *et al.*: Adv. in Int. Med., 1956, *8*, 221.
[3] RIST, N.: Unpublished data.
[4] MITCHISON, D. A.: Brit. M. Bull., 1954, *10*, 115.
[1] FJELDE, A.: Nature, 1955, *175*, 434.
[2] FJELDE, A.: Cancer, 1955, *8*, 845.
[3] FJELDE, A.: Experimental Cell Research, 1956, *10*, 88.
[4] FJELDE, A.: In press and unpublished material.

using different strains of tubercle bacilli (human, bovine, BCG, and avian) and saprophytes.

Mycobacterium strains tend to carry over into tissue culture the characteristics which are theirs in synthetic medium, i.e., the tendency to form cords, growth rate, et cetera.

It can be stated that all of the tubercle bacilli investigated, including virulent, attenuated, BCG, and freshly isolated strains from patients, grew in direct association with the tissue culture cells when a low number of tubercle bacilli was used for inoculation. When a heavy inoculum of tubercle bacilli was used, this relationship was not apparent.

In contrast, saprophytic mycobacteria grew in the fluid and on the clot and only accidentally on the culture.

A mycobacterium (No. 2357) which was isolated from a patient with a disease indistinguishable from tuberculosis behaved in tissue culture in a decidedly different way from any of the strains of tubercle bacilli investigated. The organism grew rapidly in the fluid and on the clot, thus resembling the saprophytes. This organism did not cause disease in rabbits or guinea pigs, and in regard to growth on artificial media resembled a saprophyte.[5]

When inoculated in low numbers, all strains of mycobacteria caused the mammalian cells to grow more vigorously and to appear morphologically in better condition than the controls during the initial period of infection.

A more detailed report of these investigations is in preparation.

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Yours sincerely,
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Hans Chr. Engbaek

September 4, 1956

[5] Engbek, H. C., *et al.*: To be published.

Books

SEGMENTAL ANATOMY OF THE LUNGS. By Edward A. Boyden. Pp. 276, McGraw-Hill Book Company, Inc., New York, 1955. Price, $15.00.

A collection of previously reported articles by the author, supplemented by as yet unreported material, makes this book the most complete and definitive description of segmental anatomy yet written. Its mass of detail detracts from its interest for students and those not specializing in chest diseases, and for this reason additional simpler illustrations would have added to its value. None the less, it is an excellent reference for all.

Of special interest are the chapters on development and gross anomalies. Here the author's extensive knowledge is clearly demonstrated by his ability to describe the anomalies simply and clearly, both from the point of view of their development and of what may be encountered by the clinician. Here he shows that one cannot predict either the exact order or the exact place in which a given segmental bronchus will first appear. He points up the innumerable variations that may exist—a warning to the surgeon that, although there are prevailing patterns, the most striking feature is the number of variations!

Available information on lung growth after birth is nicely summarized to show that it is not completely explained as yet. Whether or not the alveoli increase in number is not settled and awaits further examination of changes in childhood. It is suggested that new work with the electron microscope may solve the nature of the little understood alveolar membrane.

The author gives a lucid description of the congenital anomalies which include agenesis of the lung, the left aparterial bronchus, the lobe of the azygos vein, and the accessory pulmonary arteries associated with congenital cysts of the lung. Of particular interest is his clarification of the reported inconsistencies concerning the relation of accessory pulmonary arteries to congenital cysts of the lung. The logical explanation for their development leads to the following conclusions: that the anomalous artery and the cystic condition are not related as cause to effect; that the cyst may compress the artery rather than the reverse; that whatever condition produces the one anomaly is also favorable to the formation of the other.

This book is a valuable addition for library reference and is recommended to those particularly interested in pulmonary disease.

CRANSTON W. HOLMAN
New York, New York

TUBERCULOSIS IN OBSTETRICS AND GYNECOLOGY. By George Schaefer. Pp. 307, Little, Brown and Company, Boston, 1956. Price, $8.75.

Tuberculosis in Obstetrics and Gynecology by George Schaefer is a very fine treatment of, and a reliable guide for, anyone interested in this subject. The book would be of value to gynecologists, physicians in general practice, and to students and house staff. Dr. Schaefer has an expert's knowledge of the subject of tuberculosis and throughout his book he stresses the natural course of the infection as more important than the effect of pregnancy or surgery.

The book is a thoroughly practical one and completely up to date concerning concepts of treatment for tuberculosis. Details regarding drug dosage, drug combinations, and the

indications for collapse treatment and resection are discussed. Case reports of patients who have undergone extensive chest surgery for tuberculosis and who have successfully negotiated pregnancy and delivery are interesting and instructive.

Tuberculous infection of the pelvic organs and genitalia are thoroughly discussed and the respective place of surgery and of chemotherapy is set forth in detail.

The book is well written, the bibliography large, and the illustrations well reproduced. This book is, in every way, one that can be highly recommended.

WILLIAM R. BARCLAY
Chicago, Illnois

BOOKS RECEIVED

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Obituaries

CHARLES OSCAR GIESE

1876–1956

Dr. Charles Oscar Giese, a representative director to the National Tuberculosis Association from Colorado during the period 1935–1940, died at his home in Colorado Springs of arteriosclerotic cardiac disease on the morning of April 24, 1956. A graduate of the St. Louis University Medical School in 1905, he settled in Colorado Springs in 1910 and there established his practice.

At one phase of his career, he was medical director of the Modern Woodmen Sanatorium in Woodmen; he was also a member of the medical staffs of the Memorial, the Glockner-Penrose, and the St. Francis hospitals. He was an active member of the American Trudeau Society. Helping to found the American Academy of Tuberculosis Physicians, he was its first president. The Colorado Tuberculosis Association absorbed a considerable amount of his interest; among the offices he held in it was the presidency. Other medical memberships included the American Medical Association, the Colorado Medical Society, and the El Paso Medical Society.

Dr. Giese's clinical experience was spread over the years when tuberculosis was ceasing to be an unconquered scourge and was being made more and more amenable to the control of the practicing physician. Despite his deep preoccupation with the tuberculosis movement in its broadest aspects, he found time a decade ago to concern himself with efforts to prevent poliomyelitis.

His eighty years of life were actively spent; there remain many grateful ex-patients to mourn his passing and to recall with gratitude his help in their recovery from the White Plague.

S. J.

WILLIAM SIEGAL

1891–1956

Dr. William Siegal died on August 30, 1956, at his home in McKownville, near Albany, New York. He was sixty-one years of age.

Dr. Siegal was a native of Austria and came to the United States as a young boy. He was graduated from New York University and Bellevue Medical College and in the next few years was a resident physician at the Maryland Tuberculosis Sanatorium and a physician in the New York City Health Department. He joined the New York State Health Department in 1928 as a clinic physician and two years later was made Assistant Director of the Tuberculosis Division. He soon became Director of the Tuberculosis Case-Finding Bureau, and helped to establish mass chest roentgenography in the State of New York. He was considered a national authority in this field and retired from the state position in May, 1955.

In addition to the American Trudeau Society, Dr. Siegal belonged to the County, State, and American medical associations, the American Public Health Association, and the American Board of Preventive Medicine. He was also an officer of the State Civil Service Employee's Association.

Dr. Siegal is survived by his wife Pearl, a son David, a daughter Ellen, two brothers, and a sister.

W. H. O., Jr.

P. E. A. NYLANDER

1897–1955

Per Edvin Alfred Nylander, Professor of Surgery at the University of Helsinki, Finland, died suddenly of a heart attack on April 14, 1955, while attending the Congress of the German Surgical Association.

Dr. Nylander was born in Helsinki on December 11, 1897. He attended the University of Helsinki, became a Licentiate in 1924, and received the degree of Doctor of Medicine in 1927. He was appointed docent in surgery in 1930 and progressed through several ranks to the regular professorship of the department in 1943. He obtained higher honors in his own school and visited and studied in Germany, Great Britain, Italy, and the United States through the succeeding years.

Early in his career, Professor Nylander was particularly attracted to problems in thoracic surgery and studied in Professor Sauerbruch's famous clinic. He became a pioneer in thoracic surgery in Finland, and later in cardiovascular surgery.

He was president of the Finnish Surgical Association between 1944 and 1947 and a member of German, Norwegian, Swedish, European, and international medical associations. He was president of the Finnish Chapter of the American College of Chest Physicians.

In addition to his teaching and many years of research work, he was an editor of several publications. He edited *Duodecim* from 1936 to 1940; he was on the editorial panel of the surgical section of *Excerpta Medica*; he was editor of the *Annales chirurgiae et gynaecologiae Fenniae* from 1945 until the time of his death. In a published obituary it was said: "When he died, Finland lost one of her great surgeons and also a great, humane personality. There are so many who mourn his death: so many friends, colleagues, pupils, and grateful patients."

W. H. O., Jr.

AMADEO VICENTE-MASTELLARI

1907–1956

Amadeo Vicente-Mastellari died in the Canal Zone, Panama, on September 9, 1956, after a prolonged illness. He is reported to have died of uremia, secondary to malignant nephrosclerosis and malignant hypertension.

Dr. Vicente-Mastellari was a graduate of George Washington University School of Medicine in 1931, and took further training in hospitals in the Canal Zone of Panama.

At the time of his death he was Chief of the Chest Service, Gorgas Hospital, Canal Zone; Director, Tuberculosis Division, Panama Department of Health; Professor of Phthisiology of the School of Medicine, National University of the Republic of Panama; and Director of the Anti-Tuberculosis Campaign of the Republic of Panama. The list of his other services is almost incredible and is a testimony to his energy and great value in his chosen field. Dr. Vicente-Mastellari was a Fellow of the American College of Physicians and a life member, as well as Governor for Central America and Panama. He was a Fellow of the American Public Health Association; a Fellow of the American College of Chest Physicians, as well as Assistant Editor of *Diseases of the Chest*. He was a member of the Panamanian Academy of Medicine and Surgery, as well as a member of the National Medical Association of the Republic of Panama and the Isthmian Medical Association. He

was a member and president of the Sociedad Centro Americana de Tisiologica, and an honorary member of the tuberculosis societies of Venezuela, Guatemala, Mexico, and Argentina. He was a corresponding member of the Cuban, Chilean, Mexican, Peruvian, Brazilian, Colombian, and Equadorian tuberculosis societies.

It is needless to say that all of the Central, South, and North American antituberculosis movements will miss him.

W. H. O., Jr.

NOTICES

TUBERCULOSIS CONTROL: PLANS FOR INTENSIFIED INTER-COUNTRY ACTION IN EUROPE. REPORT OF A STUDY GROUP

This report summarizes the discussions of a study group convened by the World Health Organization in order to enable European countries to exchange views on recent experience in tuberculosis control and on the advisability of reorienting their control programs, following the rapid decrease in tuberculosis mortality which has occurred in recent years. The group limited its discussions to pulmonary tuberculosis.

The report first examines the measures to be taken to ensure the comparability of statistical data from different countries. It recommends, *inter alia*, that a precise definition should be given for the terms "case of pulmonary tuberculosis" and "morbidity rate," which are often used with different meanings in different countries. Furthermore, whereas in the past mortality rates have sufficed for estimating and comparing the prevalence of tuberculosis, it is now necessary to have other indices, which might be obtained either from routine health statistics or from special surveys. The establishment in each country of a central tuberculosis register, carefully kept up to date, would be of great value for the epidemiologic study of the disease. Among other features, it would make it possible to obtain at all times, by an inventory of the register, details of the prevalence of bacillary cases. Should it not prove possible, at least in the near future, to organize a national register, then regional registers could be established which would be gradually extended to cover the whole country. An annex gives a list of the information which should be collected for each case included in the register.

With regard to the indices which can be obtained from special surveys, the report recommends, on the one hand, the tuberculin index and, on the other, an index based on a complete examination for tuberculosis of sample population groups. The latter index is the more reliable, provided that the groups examined include persons of all ages. If complete population groups cannot be examined, the military conscript class would appear to be the most suitable for purposes of international comparison. As to the tuberculin index, although its value is more restricted, it can be obtained in practically all countries; it should be widely used as a first step.

Among specific measures for tuberculosis control, case finding will continue to play an important part, but the method to be employed will vary according to the country. In regions with low prevalance, the examination of contacts will become increasingly necessary. The report stresses the role which dispensaries will, for a considerable time yet, have to play as diagnostic centers; in particular, they should continue to be responsible for the examination of specially exposed groups. The report gives general directions for the systematic examination of the population, covering either total population groups or special groups, such as patients admitted to the hospital, old people, and students. A suggested code for the classification of the results of examinations is given in an annex.

The report points out the value of BCG vaccination as a supplementary preventive measure, particularly for the contacts of infectious cases and for highly exposed groups. The control of bovine tuberculosis will become of increasing importance for prevention of the disease in human beings, owing to the relative decrease in the number of infectious cases in humans.

Hospitalization of patients with infectious disease will continue to be a major factor in tuberculosis control for a number of years; however, it is to be expected that in Europe the number of beds required for tuberculosis will diminish. The extensive use of new

antituberculous drugs raises the problem of the resistance of the bacilli to such drugs. Information should be collected on the frequency of new cases of infection with resistant strains by means of a systematic study of the bacilli isolated from all new cases in a limited, defined area.

Finally, the report stresses the need to encourage the use of rehabilitation facilities, the participation of physicians in the control of the disease, the education of the public, and the provision of social and economic assistance for patients and their families.

This report is published by the World Health Organization: Technical Report Series, 1956, No. 112. Available also in French and Spanish.

BCG PRICE SCHEDULE

The following information has been furnished by Research Foundation, 70 West Hubbard St., Chicago 10, Illinois, at present the only licensed commercial producer of BCG in this country:

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Vaccine (Freeze-Dried BCG)

Ampoule ..... each $3.00
(14 vaccinations—1 site)
(7 vaccinations—2 site)
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Diluent

Sterile for resuspension of freeze-dried BCG furnished without charge.
For *Intradermal* use of vaccine (4.5 ml. ampoule) ..... each $0.50

Magnet Type Holders

For use with multiple puncture vaccinating discs ..... each $3.00

Discs (Multiple Puncture)

Multiple puncture vaccinating discs ..... each $0.10
Minimal charge per order $1.00

Note: Dried BCG vaccine is shipped by airmail and postage charges are added to price of vaccine. All prices are F.O.B. Chicago.

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May, 1956

SEVENTH INTERNATIONAL CANCER CONGRESS

LONDON, ENGLAND

July, 1958

The Seventh International Cancer Congress will be held in London, England, July 6–12, 1958, under the presidency of Sir Stanford Cade. Congress headquarters will be The Royal Festival Hall.

There will be two main sessions of the Congress: (*A*). Experimental, and (*B*). Clinical and Cancer Control.

Special emphasis will be placed on hormones and cancer, chemotherapy, carcinogenesis, and cancer of the lung.

Proferred papers will only be considered if submitted with an accompanying abstract (not over 200 words) before October, 1957, and if dealing with new and unpublished work.

The registration fee for the Congress will be £10 (ten pounds) or $30 (thirty dollars) and the latest date for registration without late fee will be *January 1, 1958*.

Registration forms and a preliminary program will be available early in 1957 on application to: The Secretary General, Seventh International Cancer Congress, 45 Lincoln's Inn Fields, London, W.C. 2, England.

SIXTH INTERNATIONAL POSTGRADUATE COURSE IN FUNDAMENTALS OF THORACIC CLINICAL SCIENCE AND SURGERY

The Sixth International Postgraduate Course in Fundamentals of Thoracic Clinical Science and Surgery will be held at the University of Groningen, Groningen, The Netherlands, from May 27 to June 15, 1957.

For further information write to Dr. R. Brinkman, Secretary, Moddermanlaan 1, Groningen, The Netherlands.

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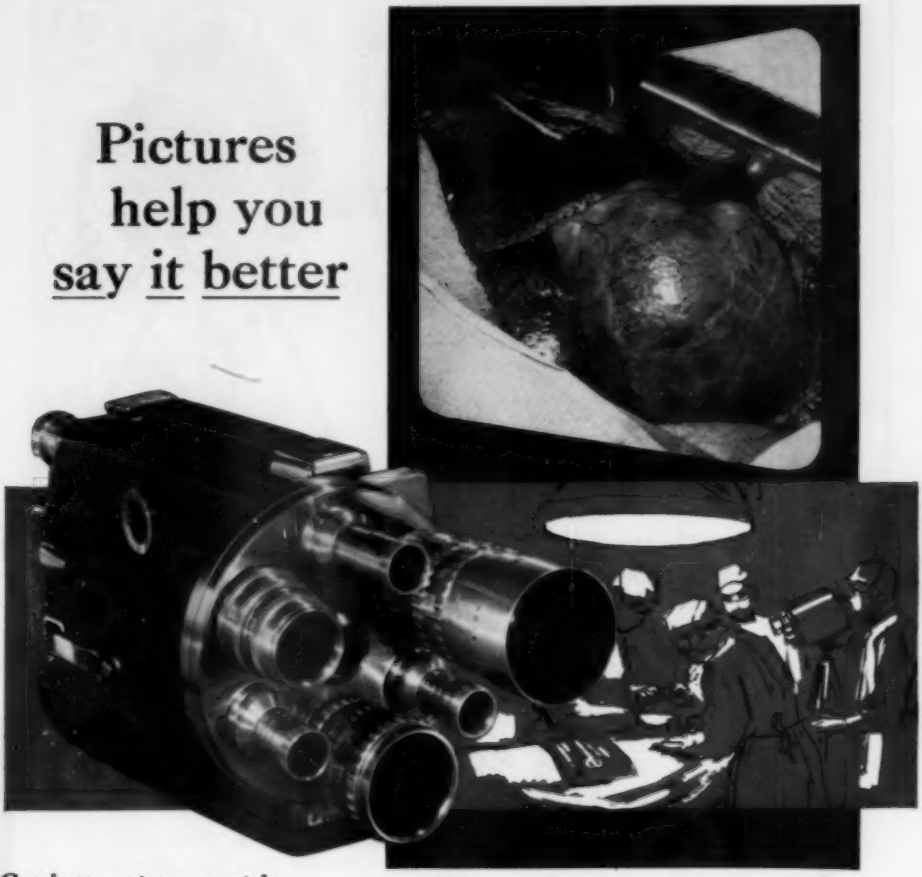

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[1]Dr. Samuel Phillips: *Calcium Benzoyl PAS*, Paper presented at 15th VA-Army-Navy Conference on Chemotherapy of Tuberculosis, St. Louis, Missouri, February 6-9, 1956.

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Forty-second Session:

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Note: The Trudeau School is supported by grant from the Lillia Babbitt Hyde Foundation, New York, N. Y.

For Schedule and other information write to

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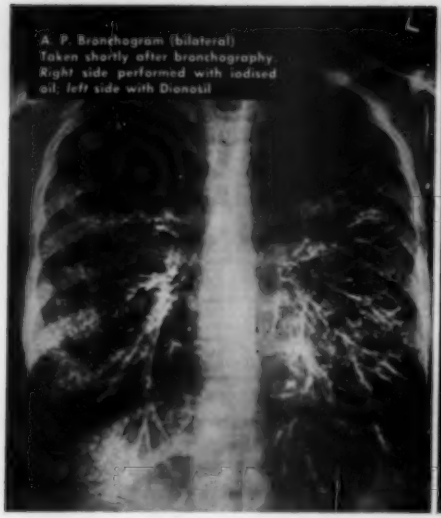
Saranac Lake, N. Y.



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2. Piper, C. E., and Nicklas, F. W.: Industr. Med. 23:510, 1954.

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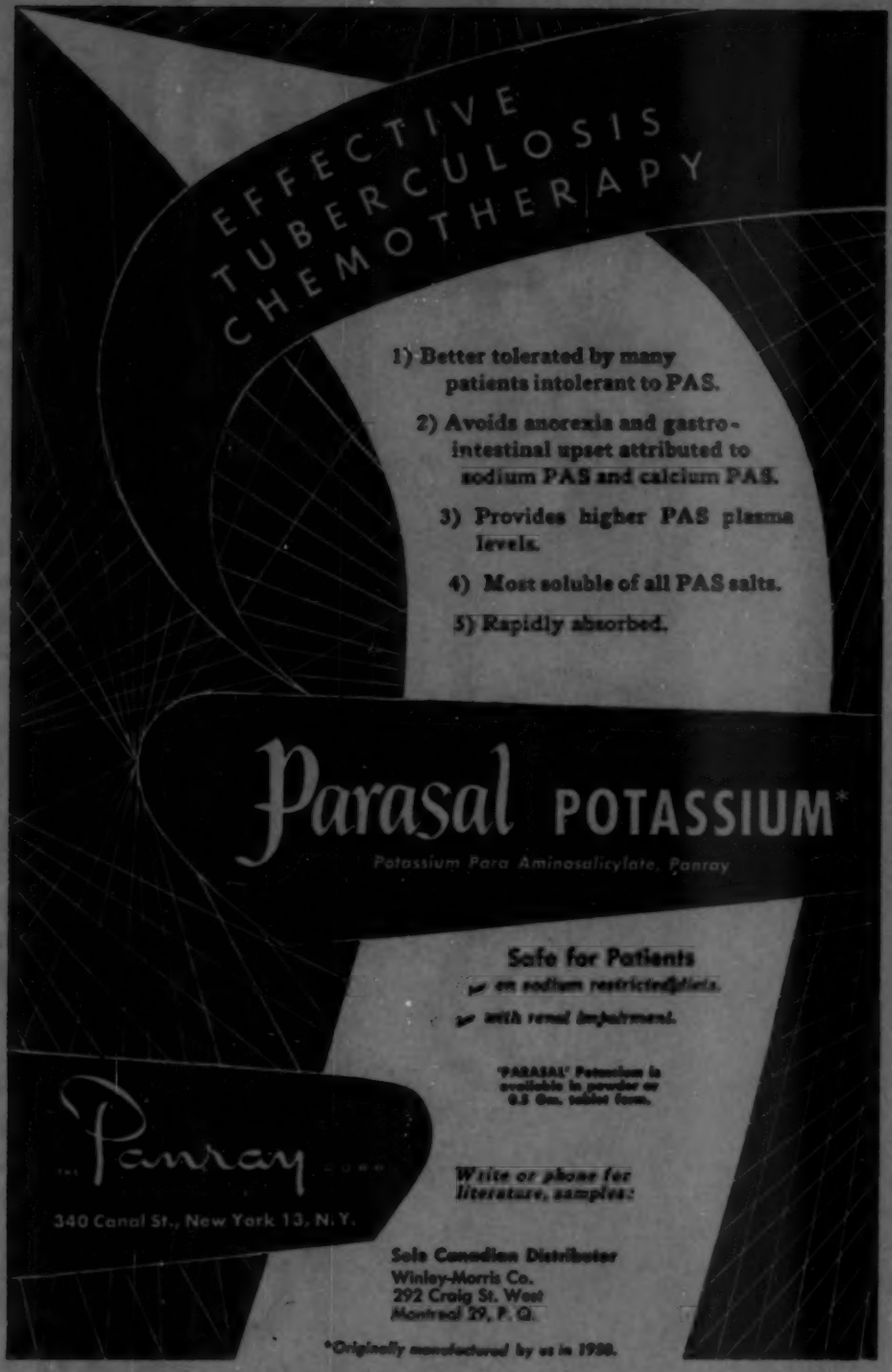
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